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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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Volume XLII.

WOODS HOLE, MASS.

JANUARY TO JUNE, 1922

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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Vol. XLII. *January, 1922* *No. 1.*

BIOLOGICAL BULLETIN

IDENTICAL TWINS IN CATTLE?[1]

JOHN W. GOWEN.

The phenomenon of a sterile, female-appearing animal resulting as a co-twin to a normal male has called attention to the problem, Do identical twins occur in cattle?

Three lines of evidence are available in answer to this question. The first consists of anatomical evidence, the ovaries of a twin-producing cow during pregnancy. This is the most critical and most desired. The second consists in the study of the normal sex ratios occurring in multiple births of cattle in comparison with those occurring in species where identical twins are produced. The third method consists in a comparison of the color markings of twins of the same or opposite sex.

In his paper on the freemartin, F. R. Lillie[2] collects data on the condition of the ovaries of cows producing twins. He showed in 22 cases where both ovaries were obtained corpus luteum were present in each. In 11 cases where only one ovary was obtained 9 had corpus luteum in that ovary. The two pairs of twins whose mothers lacked corpora lutea in the remaining ovary were of the same sex, one pair being males, the other pair females. These last two pair of twins may be identical or monozygotic twins. This appears doubtful, for as the data of Cole presented later will show, the proportion of single ovaries ovulating twice when the other one does not ovulate should not be very far from the proportion indicated by Lillie's data, providing, of course, that one ovary may ovulate twice when the other does not ovulate at all.

Lillie furnishes other data on the sex ratio of intrauterine twins beside that indicated above. The ratios which he reports for all his data are 19 male, 24 male and female, and 11 female, the methods adopted being such as to insure a random sample of the general population of cattle twins. Cole[1] has also furnished data on the sex ratios of cattle twins which he obtained from cattle breeders' records of 303 twins. The ratios of these cattle twins were 43 male, 165 male and female, and 88 females. Seven cases of triplets were also reported. Besides these statistics the Maine Agricultural Experiment Station has collected data on the frequency of the different sexes of twins as they occur in Maine.[2] These twin births were born to services for which this station had the service records. All services were traced in case the birth record did not appear at the expected time and either the proper birth record obtained, the cow found barren or the cow killed before parturition. These records should form a very homogeneous set of data in which the sex of the twins was governed only by chance. The data from these results show 5 males, 16 male and female, and 5 females. The University of Maine Animal Industry Department has always kept records of its herds since it was established. These records should be a homogeneous set of data wherein only chance was acting on the sex of the twin births as the service dates and the disposal of all animals are known. The ratio of the sex of these twin births in these data are 4 males, 4 male and female, and 3 females. These data summarized are shown in Table I.

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[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 141.

[2] Lillie, Frank R., 1917, "The Freemartin; A Study of the Action of Sex Hormones in the Foetal Life of Cattle," in *Jour. Expt. Zoöl.*, Vol. 23, p. 371–422.

TABLE I.

SEX RATIOS OF TWIN BIRTHS IN CATTLE.

Authority.	Males.	Male and Female.	Females.	*P.*	*P* for a 1 : 2 : 1 Ratio.
A. Cole	43	165	88	*AB.* 001	.045
B. Lillie	19	24	11	*BC.* 55	.29
C. This paper	9	20	8	*AC.* 25	.92

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[1] Cole, L. J., 1916, "Twinning in Cattle with Special Reference to the Freemartin," Science, N. S., Vol. 43, p. 177.

[2] The coöperative data mentioned were collected by Dr. Raymond Pearl while at this Station. The records for the college herd were obtained from the Animal Husbandry Department by the author.

The comparison of the three frequency distributions is made under *P*. The data of Cole and Lillie clearly do not correspond, the male class in Cole's frequency distribution being proportionately small, whereas the male class in Lillie's data is proportionately large. The data of Lillie and this paper correspond rather well. Here, again, the chief trouble is in the proportional differences of the male classes. The data of Cole and this paper are not so very far different, the great discrepancy being in the male class. The data of this paper are clearly about half way between those of Cole and Lillie in the proportion of sex. Cole's data were obtained from breeders. It is well known that the breeders register only about one half as many males as females in their herd books. Without question Cole would not knowingly have taken data so loaded. It is conceivable, however, that the breeders from whom he obtained his data might have been influenced by this proportion.

If identical twins are lacking in cattle, the proportion of births should be 1 : 2 : 1, providing no disturbances like lethal factors exist in the stock. The comparison of the frequency distributions to this proportion are given in the second column of *P* for Table I. No excessive deviations from this proportion exist. The poorest approach to expectation is the distribution presented by Cole. Here, judging from the male and female twins and the female twins, the male twins should be more frequent. This evidence is opposed to that of expectation on the basis of the production of identical twins.[1] Thus in human twin births, where identical twins are produced, the proportions of sex are: males 234,497, male and female 264,098, females 219.312, according to Nichols.[2] This proportion corresponds to a ratio of 1.07 : 1.20 : 1. In species where identical twins are produced it is clear that the like sex should be produced more frequently than the unlike sex of twins, whereas in Cole's data the like sex, the males in particular, are in defect. The proportion of twins as given by Lillie's data is more nearly in accord with the expectation of some identical twins. However, it will be noted that the male class is in excess and the female class in defect of its expected ratio. The agreement between the proportions of twins for these data and the proportions of twins on the theory that they are distributed in accord with those of human twins, where identical twins are known to exist, shows a slightly poorer agreement ($P = .24$) than does the theory of 1:2:1. The data of this paper clearly show a distribution of 1:2:1, indicating that identical twins are seldom produced in cattle.

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[1] The explanation of Cole for this difference will be taken up later.

[2] Nichols, J. B., Memoirs of the American Anthropological Ass., I (1907), also in Newman, H. H., "The Biology of Twins," Univ. Chicago Science Series.

Independent confirmation of this hypothesis may be obtained. The American Jersey Cattle Club describes the animals registered. From the Herd Books of this Association 749 twin females, 168 twin males and 207 male and female were taken for a comparison of their color markings. These twins have been taken and their white markings, tongue color and switch color recorded. The by-laws of this Society in 1913 do not mention any requirement to indicate that the female twin can not be registered before she is proved a breeder. Besides these, 523 pairs of females, the offspring of the same sire and dam, have been tabulated for these colors. These last were found from the Registry of Merit tables in conjunction with the Herd Books. Table II. shows the distribution for these data.

Table II.

	Female Twins.		Male Twins.		Male & Female Twins.		Sister and Sister.	
	Absolute.	Percentage.	Absolute.	Percentage.	Absolute.	Percentage.	Absolute.	Percentage.
Alike in all particulars	449	59.95	103	61.31	117	56.52	300	57.36
One body mark different	22	2.94	8	4.76	5	2.42	19	3.63
Many body marks different	43	5.74	7	4.17	15	7.25	35	6.69
Tongue color different	36	4.81	12	7.14	15	7.25	33	6.31
Switch color different	20	2.67	7	4.17	8	3.86	12	2.29
Tongue and body color different	22	2.94	3	1.79	4	1.93	16	3.06
Switch and body color different	57	7.61	10	5.95	12	5.80	20	3.82
Switch and tongue color different	25	3.34	5	2.98	7	3.38	15	2.87
Different in all particulars	75	10.01	13	7.74	24	11.59	73	13.96
Total	747		168		207		523	

Table II. shows that the percentage of cows which are alike in their body markings, tongue color and switch color are approximately the same for the four groups of cattle. The greatest difference is between the twins which are the males and the twins which are the male and female. Here the difference favors the like sex twins by about 5 per cent. It will be noted that the like sex twins resemble each other between 3.43 and 4.87 per hundred more than do the twins which are male and female. If the like sex twins are compared for resemblances with the sister, sister combination of column five, the difference becomes less. These facts all point to not more than a low percentage of identical twins in cattle if they exist at all.

If the differences are compared and their significance determined, it will be seen that none of the combinations, female twins, male twins, male and female twins or sister and sister, are significantly different. In fact, the differences are at no time greater than two times the probable error of such difference. If the whole distributions are compared by the χ^2 method, it is seen that they resemble each other closely. Such being the case, it can not be argued that twins of identical markings, tongue color or switch color exist in cattle.

In the Armadillo,[1] *Dasypus novemcinctus,* when the 4 offspring are known to be of monozygotic origin it has been shown that the degree of resemblance between the members of the quadruplet is very high. This resemblance is nearly twice as great as that which exists between brothers of known dizygotic origin. It is consequently fair to assume that the production of identical twins, in the sense that they arise from a single egg, will increase the proportion of identical offspring in like sex twins as compared with twins of unlike sex, or sister and sister combination. The above data have shown that the proportion of like sexed twins do not differ appreciably in the proportions which resemble each other from those which are of unlike sex or of sister, sister combination. Such being the case, it follows that identical twins (monozygotic) are rarely or never produced in cattle.

The importance of this conclusion as related to the freemartin problem has previously been discussed by F. R. Lillie[1] and need only be mentioned here. Hart, Cole and others have assumed that the freemartin is an undifferentiated male formed from the division of a single male-producing egg. Clearly, on this basis, these animals should be identical in appearance and the identical classes in the groups female twins, male twins and male and female twins should be large compared with those of the sister, sister class. As this is not so, it becomes doubtful if the hypothesis adopted by them will hold for the origin of the freemartin.

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[1] *Loc. cit.*

INHIBITION OF LOCOMOTION IN PARAMECIUM AND OBSERVATIONS ON CERTAIN STRUCTURES AND INTERNAL ACTIVITIES.[1]

CHARLES E. BILLS,

Zoölogical Laboratory of the Johns Hopkins University.

The study of *Paramecium* under magnifications sufficiently high to permit of observations on the activities within the living organism has been considerably hindered in the past by the excessive motility which this protozoön exhibits. A number of methods, physical and chemical, have been employed in attempting to overcome this difficulty; yet all of these methods are open to the objection that they are transient, or that they hinder the observation of certain activities, or even that they are severely limited in their application by the sensitiveness of the organism to protoplasmic poisons and osmotic changes.

Of the physical methods for quieting paramecia the following may be mentioned: (1) entanglement in zoöglea or other debris; (2) retardation of locomotion in gelatinous media; (3) inactivation at low temperature. The objections to these methods are patent: Entanglement affords an occasional arrest of activity; but in the infrequent cases where it is by chance efficient the animal is often obscured from view. Retardation in such media as quince seed jelly or gelatin is effective only when the gel is sufficiently thick to prevent the entrance of food particles at the peristome, in which case observation on this activity is impossible. Chilling may greatly reduce locomotion, but internal movements are at the same time unavoidably retarded.

The chemical narcotizing agents in general use for the lower animals are also in certain respects unsatisfactory for *Paramecium:* Methyl alcohol and chloral in concentrations sufficiently high to give any noticeable effect render the animal abnormal in appearance before paralysis sets in. Ethyl alcohol is open to the same objection, though to a less degree. Chloretone in concentrations around one tenth per cent. is serviceable; however, the margin between the narcotizing and lethal doses is so small that great precautions are necessary in its use.

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[1] This paper was prepared under the direction of Professor S. O. Mast.

In studying the action of several of the aliphatic alcohols the writer has observed that in propyl and iso propyl alcohols of certain dilution a balance of effects obtains such that the external (locomotor) cilia of *Paramecium* are inactivated before the internal activities of the animal are changed. Locomotion, therefore, can be reduced or stopped without appreciably affecting any of the visible activities which obtain within the animal itself.

In narcotization a single paramecium is added to a drop of the alcohol solution; or, better, one volume of a rich culture of mixed races is quickly and intimately combined with one volume of the alcohol of double the desired strength. For class demonstrations a number of cubic centimeters should be prepared by the latter method half an hour in advance and distributed to the students for study. The narcotized protozoa may then be observed under a cover glass or directly with a water immersion lens. When the animals come in contact with the stronger alcohol a few are immediately killed, while the majority at first exhibit avoiding reactions—they move erratically, become frequently bent in at the peristome, and may discharge a few trichocysts; then, as these reactions to the new stimulus cease, they become paralyzed and settle to the bottom. This final effect obtains in approximately half an hour from the application of the alcohol, the time varying with the race and condition of the individuals. The completely paralyzed paramecia exhibit little or no ciliary activity on the body, yet the cilia in the oral groove and at the peristome maintain a vigorous and coördinated movement; the undulating membrane of the œsophagus continues to direct the ingestion of food in approximately the normal manner; and the activities of the contractile vacuoles, food vacuoles and streaming protoplasm remain apparently normal for an extended period. With careful manipulation the narcosis can be continued for four or five hours, and these activities can be *gradually* retarded by heavy or prolonged narcotization. Thus it is evident that the study of *Paramecium* is greatly facilitated by the use of propyl alcohol as a narcotizing agent.

Paralysis is induced by the action of the alcohol on the cilia.

These may be inactivated, incoördinated or cytolyzed. If the narcotized animals are removed to a normal medium, those of them which are not too severely affected slowly recover. Recovery is indicated by a beginning of locomotion accompanying the reappearance of the body cilia. Whether these are actually regenerated or merely reëxtended and stiffened for swimming has not been determined.

Table I.

Showing the Optimum Concentrations of Propyl Alcohol Required to Inhibit Locomotion in Eight Pure Lines of *Paramecium*.

The percentages by volume are estimated from the conversion equivalents for ethyl alcohol.

Number of Culture.	Normal Propyl Alcohol.		Iso Propyl Alcohol.	
	Per Cent. by Weight.	Per Cent. by Volume.	Per Cent. by Weight.	Per Cent. by Volume.
P. caudatum 1	1.0	1.3	2.0	2.5
2	1.5	1.9	2.5	3.1
3	2.0	2.5	3.0	3.8
4	2.0	2.5	3.0	3.8
5	2.5	3.1	3.0	3.8
P. aurelia 1	2.0	2.5	3.5	4.4
2	2.5	3.1	3.0	3.8
3	2.5	3.1	3.5	4.4
Average	2.0	2.5	2.9 +	3.7

It appears that the permissible variation from the optimum narcotizing concentration is greater with iso and normal propyl alcohols than with any of the quieting agents heretofore employed. With different cultures, however, the narcotizing concentration varies considerably, as the results presented in Table I. indicate. This table gives the optimum concentrations of the two propyl alcohols when used on various typical pure line cultures of *Paramecium caudatum* and *Paramecium aurelia*. It should be noted that the former species narcotizes more satisfactorily than the latter, and that wild cultures, or mixed cultures of pure lines, are more satisfactory than single pure lines of either species, since among the varied specimens of the mixed "populations" a considerable number which will narcotize properly can be much more readily found than in the more uniform specimens of the pure stocks.

By appropriate concentrations of propyl alcohol other microscopic animals can be narcotized, though with less advantage than *Paramecium*. *Paramecium bursaria* is paralyzed, but cyclosis also is stopped. The erratic movements of *Oxytricha* continue, though less frequently and energetically. *Spirostomum* gives acute avoiding reactions, twisting and bending so sharply at the peristome that the body is frequently severed. The cilia on the fragments continue to beat as long as any ectoplasm remains, but those on unbroken animals that are narcotized quickly disappear. *Stentor* becomes quiet, and some of the cilia at either extremity continue to beat; it contracts so much, however, that little can be seen within. *Colpidium* and *Colpoda* become quiet, but the internal activities also cease. The contractile flagellates—*Euglena, Peranema* and *Distigma*—are stimulated to pronounced euglenoid contortions; their flagella are frequently lost, and may be grown again in fresh media. In the case of *Peranema* the writer has observed the flagellum actually regenerate about one fifth its length in three hours. Of the rotifers, *Monostyla* narcotizes satisfactorily; many of the others, however, merely undergo contortions up to the point of death. The various larvæ and nematodes that were observed gave likewise results of no special interest. In general, then, it may be said that while propyl alcohol gives a more or less effective narcotization in a number of microörganisms, *Paramecium* is the only form studied in which internal activities of a strikingly obvious character obtained after narcotization.

Some paramecia, narcotized in accordance with the technique above indicated, were examined in detail. It was ascertained that narcotization greatly simplifies the study of familiar details—cyclosis, functioning of the vacuoles, structure and activities of the œsophagus, and feeding. Several new observations and corrections were made; these are indicated below with the topics to which they refer.

In a narcotized paramecium, lying with the oral groove up, and the anterior end pointing to "twelve o'clock," there can be seen a steady and vigorous cyclosis extending anteriorly along the right fold of the body (anterior stream) and posteriorly along the left fold (posterior stream). The writer noticed that the cyclosis may at times accelerate at some part; this acceleration is local and limited and soon traverses the entire course, ceasing first where it began. The acceleration seems independent of the functioning of the contractile vacuoles, since it occurs at intervals much greater than those of vacuole closure. The contractile vacuoles, fixed to the ectosarc, can be seen to operate, as in normal animals, at intervals of from ten to twenty seconds.

The gullet of *Paramecium* is usually represented in the literature as a short and narrow tube tapering posteriorly to the region of the aggregation of food particles, at which position it expands into a sac of somewhat greater diameter than the gullet proper. This description is doubtless referable to the fact that in the dead animal the œsophagus presents an appearance quite different from that in the living. In specimens carefully killed, fixed and stained the gullet was found to be scarcely recognizable, although the other structural details seemed unimpaired. In animals freshly killed, but not otherwise treated, the gullet presents the appearance indicated in the literature. The writer has often observed that in paramecia being killed by dilute poisons a convulsive rearrangement in the protoplasm in the posterior half of the body chokes up the œsophagus and tends to elevate the oral depression. The gullet, therefore, shortens and constricts, and if a new food vacuole is aggregating at this time the distal end of the gullet naturally remains relatively larger than the shrunken duct itself.

In a narcotized living paramecium the gullet can be observed as a smoothly curved and posteriorly tapering tube, approximately one fourth the length of the body. It extends from the peristome posteriorly toward the aboral side, but not through the middle of the body, so that the distal end lies near the ectosarc; and as seen from the oral surface and the posterior end, it twists obliquely to the left of the longitudinal axis of the animal (Fig. 1, *a*, *b*, *c*).

The undulating membrane, or ciliated lining of the œsophagus, was superficially studied in a few animals which had stopped swimming, but still rotated feebly, revealing the gullet in all its aspects. In these animals the undulating membrane was still functioning rapidly, though apparently somewhat slower than in a normal paramecium. The undulatory motion can be likened to that of attached ribbons blown in front of an electric fan, or to that of the succession of waves obtained by shaking and twisting a number of fixed ropes. In no case, however, even at the moment of the cessation of undulatory motion, was it possible to observe in the gullet the cilia themselves; only their wave-like motion could be

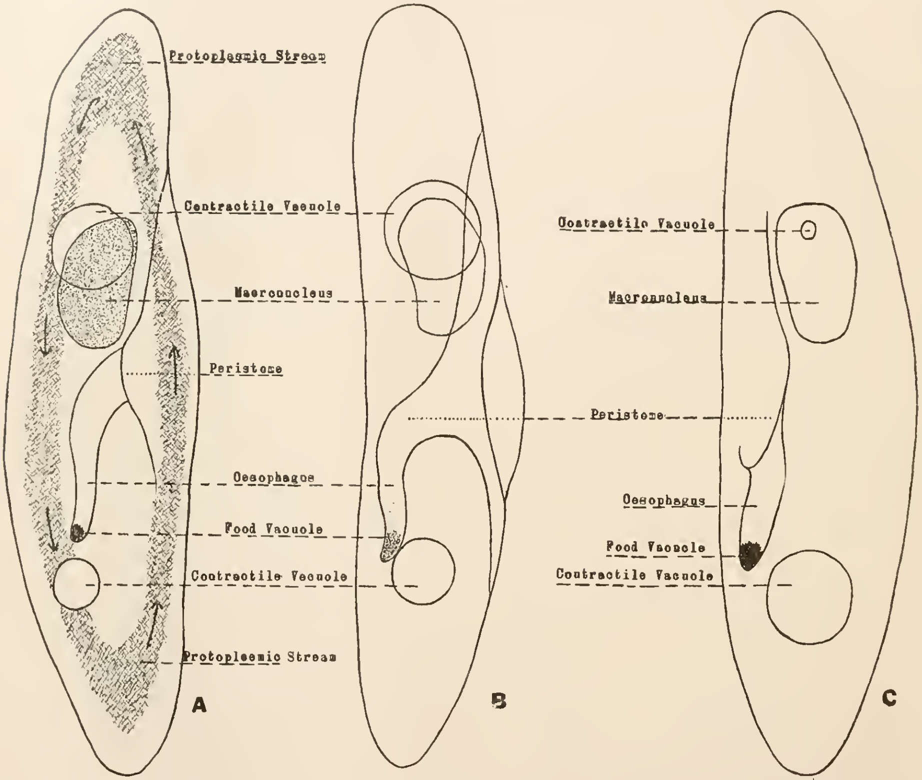
Protoplasmic Stream
Contractile Vacuole
Macronucleus
Peristome
Oesophagus
Food Vacuole
Contractile Vacuole
Protoplasmic Stream
A
B
Contractile Vacuole
Macronucleus
Peristome
Oesophagus
Food Vacuole
Contractile Vacuole
C

FIG. 1. Camera lucida tracings of specimens of *Paramecium caudatum* narcotized with propyl alcohol, intended to give especially the appearance of the gullet as the animal turns through 180°. The paramecia are heavily narcotized, and the incorporation of food vacuoles is beginning to be retarded.

A. View of paramecium in the position most frequently assumed when quiet. The gullet is in semi-profile. Food particles are aggregated, but remain in the gullet.

B. View of paramecium lying on its "side." The gullet is in full profile. Food particles are not aggregated.

C. View of paramecium from the aboral side—turned through 180° with reference to the position in (*A*). Food particles are aggregated, but remain in the gullet.

seen. The activities of the undulating membrane, in directing the passage of "food" particles (Chinese ink) to the vortex at the distal end of the gullet, and in returning unaccepted particles to the peristome, are rendered easy of observation by the quieting action of propyl alcohol. It may be, however, that the normal process of food acceptance is modified by the drug when sufficient of the latter is employed to effect a complete narcotization.

SUMMARY.

1. Normal and iso propyl alcohols, in concentrations around two and three per cent., respectively, are superior narcotizing agents for *Paramecium*.

2. In narcotized paramecia it is possible to observe with convenience the structures and activities characteristic of the organism.

3. The cyclosis is not a uniform flow, but is subject to waves of acceleration.

4. The œsophagus is a much more extensive structure than is generally represented in the literature.

THE EFFECTS OF CARBON DIOXIDE ON THE CONSISTENCY OF PROTOPLASM.

M. H. JACOBS,

University of Pennsylvania.

Introduction.

It has been shown by a number of recent investigators, using the microdissection and other methods, that the consistency of the protoplasm of different cells may vary from that of a liquid almost as mobile as water to that of a solid gel. It has also been shown that within the same cell at different times there may be almost equally striking differences. For example, Bayliss ('20) has reported that when the ectoplasm of an *Amœba* is observed with the ultramicroscope it appears to be filled with fine brilliant particles in rapid Brownian movement—an indication of the liquid state. On electrical stimulation the movement suddenly ceases, to be resumed when the stimulation is discontinued. Evidently in this case stimulation is attended by a solidification, or at least by a very considerable increase in the viscosity of the protoplasm. Another important example is furnished by cells in the act of division. Heilbrunn ('15) and Chambers ('17), using entirely different methods, have both shown that during the cleavage of marine ova there is with each cell-division a regular cycle of changes leading from a more liquid to a more solid condition and back again. It seems very likely that these changes play an important part in the actual mechanics of the division process. A third example is furnished by various cases of amœboid movement, where changes in protoplasmic consistency are thought by some of the most recent workers on the subject (especially L. Loeb, '02, '20, '21, etc.) to be concerned in actually producing the movement. A considerable number of other cases, some definitely established, others merely probable, could be mentioned. It seems certain that changes in protoplasmic consistency are not only of widespread occurrence, but are also of great physiological importance.

As to the cause of these changes, little is known. Heilbronn ('14) and Heilbrunn ('20) have shown that such effects can be produced by changes in temperature, but evidently this factor is not an important one in the cases that have been mentioned. Szücs ('13) showed that aluminium salts could bring about a solidification followed later by a liquefaction (both reversible) in plant cells, while Heilbronn, and especially Heilbrunn, have obtained striking results with ether, chloroform and other anæsthetics. Important as are these various studies on the effects of chemicals in throwing light on the nature of protoplasm, they give little information as to the cause of the physiological changes in viscosity observed under natural conditions, since the substances employed have not been such as are usually encountered in living cells.

In the course of some work done on a different problem several years ago the author observed certain effects of carbon dioxide on the protoplasm of living Protozoa which suggested the desirability of testing to what extent this substance might be able to cause reversible changes in protoplasmic viscosity. If it could be shown that it does, in fact, possess such powers in any striking degree, the observation would be a suggestive one, since CO_2 is not only a universal product of cell activity, but it has been definitely shown in certain cases (Lyon, '04) to be produced at different rates at different times within the same cell, and a plausible hypothesis might in that event connect the observed changes in viscosity, in part at least, with varying rates of CO_2 production. In the present paper evidence is presented that changes of the sort demanded by such an hypothesis may, as a matter of fact, be produced.

The material used consisted of the two Protozoa, *Paramœcium caudatum* and *Colpidium colpoda*, the eggs of the sea-urchin, *Arbacia,* and in a few experiments filaments of *Spirogyra*. In addition, a number of observations were made on *Amœba*. The consistency of the protoplasm of all of the cells except *Amœba* was tested by the centrifuge method of Szücs and of Heilbrunn, advantage being taken of the fact that the more viscous the protoplasm is, the more difficult it is to bring about a separation from it by means of centrifugal force of solid bodies of different specific gravity contained in it. The cells studied either contained bodies capable of responding to this treatment (pigment and other granules and droplets of *Arbacia* eggs, chloroplasts of *Spirogyra,* certain fine granules in *Colpidium*) or such bodies could be introduced in the form of food vacuoles filled with India ink (*Paramœcium* and *Colpidium*). The force used with the *Arbacia* eggs was estimated at 1,600 times and with the other material at approximately 150 times that of gravity. The *Arbacia* eggs were centrifuged in sea water, *Spirogyra* in pond or in distilled water, and the Protozoa for the most part in their own culture fluid.

A portion of the experiments here described were carried out at the Marine Biological Laboratory at Woods Hole. The author wishes to acknowledge his indebtedness to the Director, Professor Frank R. Lillie, for placing at his disposal the facilities of the laboratory during a part of the summer of 1920.

Experiments on Paramœcium.

The procedure most frequently employed with this form was as follows: The animals were first placed in a test tube with about 20 c.c. of culture fluid to which a few drops of a dense suspension of India ink had been added. They were then allowed to stand for 30 minutes, at the end of which time they contained numerous black food vacuoles whose behavior could be followed with the greatest ease. Before exposure to carbon dioxide they were tested to determine the ease with which the food vacuoles could be separated from the remainder of the protoplasm. It was found in all the experiments with the culture used that a two minutes' exposure to a force of 150 times gravity caused a partial separation—*i.e.,* there was a general tendency for the ink to move toward the posterior end of the body—but it did not by any means become massed there, and in some individuals no movement at all was evident. When, at the end of 30 minutes, the animals had formed sufficient food vacuoles, a current of carbon dioxide was allowed to bubble through the liquid containing them, and samples were removed at intervals, immediately centrifuged for the same time and at the same rate as before, and then examined in Syracuse watch glasses. After this examination the watch glasses were in each case exposed to the air to allow the escape of the CO_2, and after several hours another examination was made to determine whether or not deaths had occurred. A considerable number of experiments were made on *Paramœcium*, and as they all showed a very satisfactory agreement in their general results, it will be sufficient to describe in detail only the following typical one.

The animals used in this experiment responded to centrifugalization before treatment with CO_2 in the usual manner—*i.e.*, by a partial, but by no means complete, separation of the food vacuoles from the protoplasm when the force employed and the time of exposure to it were as already mentioned. After the current of CO_2 had flowed for one minute a slight increase in the readiness with which separation occurred was noted; this had decidedly increased by the end of 5 minutes, and by the end of 10 minutes it had become very striking. The India ink after centrifugalization was now invariably found in a compact mass in the posterior end of the body, so closely packed that the outlines of the individual food vacuoles could scarcely be distinguished. The animals themselves at this stage of the experiment showed no visible injury either from the exposure to the carbon dioxide or to the centrifugal force. When placed in open watch glasses they not only continued their locomotion in a normal fashion, but the food vacuoles within a few minutes had become redistributed throughout the cell and the animals had resumed their original appearance.

After 15 and 20 minutes, respectively, from the beginning of the experiment the results were essentially the same as those at the end of 10 minutes, except that a swelling of the body was now becoming increasingly apparent. At the end of 25 minutes a new appearance could be noted. In a few of the individuals, although the treatment was the same as before, the food vacuoles failed to separate. The numbers of such animals gradually increased during the next 15 minutes, until at the end of 40 minutes it was the exception rather than the rule for such separation to occur, and 5 minutes later only a few showed any movements of the food vacuoles at all. At 50 minutes this condition had become practically complete, and the protoplasm of the animals was now evidently considerably more viscous than at the beginning of the experiment. This increased viscosity—or perhaps solidification—

was also made apparent by the cessation of all movements of the contractile vacuoles. Swimming, however, continued, though at a slower rate than before.

All of the effects so far described were found to be completely reversible. Even after the protoplasm had passed through its period of liquefaction and had become much more solid than at the beginning of the experiment the animals practically all recovered normally when the CO_2 was allowed to escape. From 56 minutes onward, however, evidences of fatal injury began to appear. The animals exposed for this length of time showed a mortality of about 25 per cent., though those that did not die recovered entirely normally. By the end of 65 minutes the mortality had increased to approximately 75 per cent., and 10 minutes later to 100 per cent. Since solidification began to be evident in some individuals in 25 minutes and was present in nearly all in 40 minutes, and since scarcely any deaths occurred before 50 minutes, it is evident that there is a considerable period within which the solidification is completely reversible. With an exposure of longer duration, however, it apparently merges imperceptibly into an irreversible death coagulation.

The effects so far described are much more rapidly produced if the animals have been kept in distilled water for a time before their exposure to carbon dioxide. For example, in one experiment a certain degree of liquefaction was produced in animals in the normal culture medium in 12 minutes; in individuals from the same culture which had previously been washed in distilled water this point was reached in less than 6 minutes. The times for the beginning of solidification in the two cases were 40 and 12 minutes, respectively, and while the animals in the culture medium were still living at the end of 60 minutes, those in the distilled water were dead in 32. These differences are perhaps partly due to the greater hydrogen-ion concentration of the distilled water, but experiments that need not be described here have shown that this is not the only factor concerned. The situation is, in fact, a complex one, presenting a number of points of interest, and its fuller discussion in another paper is contemplated.

At this point it may perhaps be well to consider two questions that might justly be raised in connection with the facts that have been mentioned. The first is whether the behavior of *Paramœcium* may not be due to the removal of oxygen by the current of CO_2 rather than to the action of the latter substance itself; the second is whether the changes in the readiness of separation of the food vacuoles from the protoplasm may not be due to changes in the specific gravity of the latter brought about by the absorption or the giving off of water rather than to mere changes in viscosity.

As to the first question, it may be said that a vigorous current of hydrogen, which must remove the oxygen at least as effectively as the slower current of CO_2, was not found to bring about the sequence of changes described, even when allowed to flow for a considerably longer time. It did, to be sure, appear after a time to favor liquefaction of the protoplasm, but not only was its action in this respect far slower than that of carbon dioxide, but in no case were evidences of subsequent solidification observed though the experiments were continued much beyond the time required for the solidifying action of CO_2. As to the second of the two questions, it may be noted that there appears to be no evident correlation (as there should be if the chief factor were a change in the specific gravity of the protoplasm) between the degree of swelling in *Paramœcium,* which is evidently due to the taking up of water, and the ease with which the food vacuoles may be separated from the protoplasm. A second and even more convincing argument in favor of the view here adopted will be given when the experiments on *Colpidium* are described.

Experiments on Colpidium.

The behavior of *Colpidium* when exposed to carbon dioxide agrees in its essential features with that already described for *Paramœcium.* There is a preliminary period of liquefaction followed by increasing reversible solidification, and finally an irreversible coagulation. *Colpidium* is, however, more resistant to CO_2 than *Paramœcium* and the time required to bring about the final coagulation is considerably greater. This difference is especially striking when the animals have first been placed in distilled water—in fact, it is so great in that case that it may be put to practical use in converting a mixed culture of *Paramœcium* and *Colpidium* into a pure culture of *Colpidium*. In case it is desired to make such a separation, it is only necessary to wash the animals in distilled water, concentrate them by gentle centrifugalization, and then introduce them suddenly into distilled water one half saturated with carbon dioxide. At the end of a few minutes *Paramœcium* will be found to be dead and *Colpidium* living; the CO_2 may then be removed from the water, a little dry hay added, and an excellent pure culture of the latter organism will be obtained.

Another minor difference between *Colpidium* and *Paramœcium* is that the former in its natural state appears to offer greater resistance to the separation of the food vacuoles by centrifugal force. While an exposure of two minutes, in the culture used, brought about a very distinct separation in *Paramœcium,* one of four minutes was only slightly effective in the case of *Colpidium.* One result of this greater difficulty in bringing about separation in the normal animals is that while it is very easy in *Colpidium* to demonstrate the preliminary liquefaction followed by an increasing solidification which finally leads to a viscosity at least as great as that at the start of the experiment, it is not possible to show conclusively, as in the case of *Paramœcium,* that this viscosity produced by CO_2 is actually greater than that in the untreated animals.

A third point of difference between the two forms is that in *Colpidium*—at least in the individuals studied—there are present, in addition to the food vacuoles, many fine dark particles scattered irregularly through the protoplasm. The effect of centrifugal force after liquefaction has occurred is to throw these particles to the anterior end of the body, while the food vacuoles containing ink go to the posterior end, giving a very distinct banded appearance to the animals, the anterior band being gray, the middle one transparent and the posterior one black. The appearance is very striking and can be seen even with a hand lens. With the centrifugal force used it was never obtained except in animals that had been exposed a moderate length of time to carbon dioxide. In individuals not exposed at all, or exposed a longer time, the banding was indistinct or entirely lacking.

This characteristic of *Colpidium* is of some importance in connection with the question already raised as to whether the differences in the ease of separation of the food vacuoles after exposure to CO_2 are due to changes in the viscosity or in the specific gravity of the protoplasm. In the case of the fine particles and the food vacuoles of *Colpidium* we are evidently dealing with two materials of different densities—one heavier and the other lighter than the protoplasm. If a change in the specific gravity of the latter through the taking up or giving off of water were alone concerned in facilitating separation, it is evident that a change that would favor the movement of the food vacuoles would hinder that of the fine particles and vice-versa. As a matter of fact, the movement of both is favored at the same time. The logical conclusion, therefore, is that a decrease in viscosity rather than a change in specific gravity is concerned, though it is by no means unlikely that changes in specific gravity—whose effects are, however, relatively insignificant—may also occur.

In concluding the section on *Colpidium* it may be worth while to present in tabular form the behavior of this animal and of *Paramœcium* in a typical experiment where both organisms were obtained from the same culture and were subjected together to the effects of CO_2.

Experiments on Arbacia Eggs.

Certain experiments, as yet unpublished, which the author has made with a different end in view on the eggs of the sea-urchin, *Arbacia,* confirm to a certain extent the conclusions arrived at in the preceding sections. The experiments were primarily intended to throw light on the relative efficiency of buffered solutions of the same pH, but containing different amounts of CO_2 in producing internal changes in cells. For the purpose of detecting such changes, advantage was taken of the readiness with which centrifugal force brings about a stratification of the materials found in *Arbacia* eggs into four layers. This appearance was first described by Lyon ('07) and has since been used with good effect by Heilbrunn in following the changes in the viscosity of the protoplasm of these eggs at different stages of division, under the effects of anæsthetics, etc.

TABLE I.

EFFECT OF APPLYING CENTRIFUGAL FORCE OF 150 TIMES GRAVITY AFTER VARIOUS EXPOSURES TO CARBON DIOXIDE.

Time of Exposure.	Paramœcium.	Colpidium.
1 minute	Slight evidences of liquefaction.	Slight evidences of liquefaction.
5 minutes	Liquefaction more distinct; body somewhat shrunken.	Liquefaction more distinct.
10 minutes	Practically all individuals show complete separation of food vacuoles.	Liquefaction very clear.
15 minutes	About the same; body beginning to swell.	Separation of food vacuoles almost complete.
20 minutes	Same; body more swollen.	Same; body somewhat swollen.
25 minutes	A few show failure of food vacuoles to separate.	Same as 20 minutes.
30 minutes	Same as 25 minutes.	Same as 20 minutes.
35 minutes	Same as 25 minutes.	A few show incomplete separation.
40 minutes	Many show no separation.	Less complete separation.
50 minutes	Most individuals show no separation.	More show absence of separation.
50 minutes	Absence of separation in practically all; recovery almost 100 per cent.	Separation absent in most individuals; recovery almost 100 per cent.
56 minutes	Same as 50 minutes; recovery about 75 per cent.	Same.
65 minutes	Same; recovery about 25 per cent.	Same.
75 minutes	All dead.	Same; many recoveries.

It is easy to demonstrate that carbon dioxide influences profoundly the consistency of the protoplasm of *Arbacia* eggs. In numerous experiments a five-minute exposure to sea water practically saturated with this gas was found to prevent stratification almost completely when the eggs were centrifuged for two minutes with a force estimated at 1,600 times that of gravity, while the controls in normal sea water showed the typical separation described by Lyon. That this result is due not to the hydrogen-ion concentration of the external medium as such, but rather to its carbon dioxide content (the carbonic acid probably penetrating the egg in an undissociated form or as CO_2 and subsequently dissociating within the cell), is shown by a comparison of the effects of sea water to which CO_2 has been added with those of a similar solution in which the same pH has been produced by adding another acid—*e.g.*, HCl,—cautiously, drop by drop, and shaking vigorously after each addition to remove the CO_2 set free from the bicarbonates by the acid.

The results of one experiment of this sort may be given here. The exposures to carbon dioxide and to centrifugal force were those already mentioned. The numerical values of the pH of the solutions are only approximate, since the "salt errors" of the indicators used for determining them were disregarded, but as the error was in each case the same for the two solutions which were being compared, the general results obtained were not thereby affected.

Table II.

Effects of Centrifugal Force on *Arbacia* Eggs Exposed for 5 Minutes to Sea Water of Different pH Values.

pH (approximate)	pH regulated by adding CO_2.	pH regulated by adding HCl and then removing CO_2.
8.0	Stratification complete.	Stratification complete.
7.0	Stratification complete.	Stratification complete.
6.0	Stratification decidedly incomplete.	Stratification complete.
5.5	No stratification.	Stratification complete.

It appears from this experiment that the effects of carbon dioxide are primarily internal and depend on the absolute concentration of this substance rather than on the pH of the solution. These results are in agreement with those obtained by the author with other material and described in previous papers ('20-*a*, '20-*b*).

The solidifying effect of CO_2 is at least to a certain extent reversible. In several experiments eggs exposed to saturated sea water for 5 minutes were divided into two lots. The first lot was tested immediately and found to be solidified, giving no evidence of stratification after centrifugalization. The second lot was allowed to stand exposed to the air in a shallow dish for 30 minutes to permit the CO_2 to escape, or in some cases fresh sea water was also added. In either event centrifugal force produced normal stratification, showing that the eggs had again returned to the liquid condition. Even the same eggs which had been centrifuged after exposure to CO_2 and had failed to stratify showed the normal behavior when the process was repeated after they had stood exposed to the air for 30 minutes.

It seemed desirable to determine whether in addition to this physical reversibility there was also complete physiological reversibility in the sense of permitting normal development to occur after solidification had been brought about. To test this point, a suspension of sperm was added to various lots of eggs after the CO_2 had been allowed to evaporate and the eggs had regained their fluidity. The results of these experiments were somewhat conflicting. In some cases no development occurred, in others there was cleavage as far as the 4- or the 8-cell stage, with perhaps irregular development beyond that point, while in still others normal development was obtained. The probable explanation of these differences was soon found to be that the exposure to CO_2 caused membrane formation (as had been noted by a number of previous observers) and subsequent development depended on whether or not conditions were favorable for complete artificial parthenogenesis. But whatever may have been the factors concerned in bringing about development, the mere fact that it occurred even in a portion of the cases where the eggs had undergone a very noticeable solidification is definite proof of the possibility of physiological reversibility.

It will be noticed that there have been described no liquefaction effects on *Arbacia* eggs. Whether or not such effects can be produced by lower concentrations or shorter exposures or both can be determined only when suitable material is again available. The experiments here described were made with another end in view before conditions in *Paramœcium* and *Colpidium* had been investigated, and the possibility of a preliminary liquefaction before solidification was not suspected. Though the experiments are, therefore, to this extent incomplete, their results, as far as they go, are in agreement with those obtained with the other kinds of material studied.

EXPERIMENTS ON AMŒBA.

The possibility of producing definite changes in the consistency of protoplasm by means of CO_2 suggested an interesting application in the case of *Amœba.* It has repeatedly been urged by L. Loeb ('02), ('20), ('21), etc., that amœboid movement is due to changes in protoplasmic consistency. Hyman ('17) has also expressed the same view. The fact that carbon dioxide has been shown to cause such changes in the cases just described, and the additional consideration that it must be produced in an amœboid cell at a rate that varies greatly with circumstances, give some grounds for the hypothesis that the agent chiefly concerned in bringing about the protoplasmic changes underlying amœboid movement might be none other than this substance.

To determine to what extent the formation of pseudopodia can be influenced by carbon dioxide, an extremely fine capillary was made through which CO_2-saturated water could be discharged in small amounts. On several occasions quiescent amœbas (belonging to a species similar to, but probably not identical with, *A. Proteus*) were found, and on gently discharging a very small amount of the solution of CO_2 against the body of the animal pseudopodia were immediately put out in the direction of the capillary. On at least eight other occasions a small amount of the solution was discharged against the hindmost portion of a creeping amœba. In every case the same result was obtained. Pseudopodia were first put out at the point where the CO_2 came in contact with the body, the currents in the other pseudopodia being for a time reversed. Then as diffusion occurred and the full effects of the CO_2 became apparent all movements ceased for a few seconds, the animals remaining, as it were, congealed in the form in which they happened to be when overtaken by the effects of the dissolved gas. This paralysis then quickly passed away and movements were resumed in the original direction. To all appearances at least the animals had undergone a local liquefaction followed by a more general solidification. It follows from these rather crude experiments that if the external application of CO_2 can produce such results, it is not unlikely that internal effects of the same sort, resulting from varying rates of metabolism in different parts of the body, could conceivably give rise to amœboid movements of the usual type, though further work will be required to determine how far such an hypothesis explains the observed facts.

EXPERIMENTS ON SPIROGYRA.

It was thought desirable to supplement the experiments on animal cells with similar ones on some type of plant cell to determine how general the results already obtained might be. For this purpose *Spirogyra* was chosen, as it had already been successfully employed in a similar fashion by Szücs in his work with aluminum salts, and preliminary experiments showed that CO_2 produces marked differences in the ease with which the chloroplasts can be displaced by centrifugal force.

The first lot of *Spirogyra* studied, which was obtained early in the winter, showed clearly both liquefaction and solidification effects just as the Protozoa had done. Unfortunately, before all of the details of these processes could be studied, the material was exhausted and no more could be obtained until the following spring. This latter material, while giving the liquefaction effect with the greatest clearness, for some reason failed to show solidification except such as was obviously associated with irreversible death changes. The experiments, therefore, leave something to be desired in the way of completeness, but since the results, as far as they go, especially those obtained with the earlier material, are in good agreement with those already described, a few of them may be mentioned briefly.

In one experiment with the winter material a number of the filaments were placed for 2 minutes in distilled water saturated with CO_2. When centrifuged for 2 minutes at the same rate as that employed with the Protozoa, and then examined with the microscope, the chloroplasts in practically every cell were found to be aggregated in dense masses at the sides or ends of the cells, according to the position of the filament during centrifugalization. Controls in ordinary distilled water showed a much less complete separation. On the other hand, in filaments exposed for 10 minutes there was practically no movement of the chloroplasts at all, their spiral form being maintained almost as well as in cells which had been fixed for a few minutes in boiling water before centrifuging.

In another experiment filaments of *Spirogyra* were exposed to a saturated solution of carbon dioxide for 2, 4, 8 and 16 minutes, respectively. The four lots, together with a control, were then centrifuged as before, with the following results: In the control some cells showed a separation of the chloroplasts, while others did not; in those exposed for 2 minutes practically all showed complete separation; in those exposed for 4 and for 8 minutes there was somewhat less separation (the amount being approximately the same as in the control), while with the 16-minute exposure there was very little separation and the filaments showed a certain apparent brittleness, being to some extent broken into pieces during their transfer to a watch glass for examination.

As to the reversibility of these effects on *Spirogyra,* it may be said that observations on this point are much more difficult to make than in the case of the motile Protozoa, and in the instances mentioned satisfactory determinations were not made. It has since been found with the more abundant material recently available that the filaments in which the exposure has been sufficient to produce decided liquefaction are not at all injured by the process, continuing to grow after the treatment in a normal manner. But whether or not recovery is possible after solidification can be determined only when *Spirogyra* which shows this behavior as clearly as did the first lot studied is again available in considerable quantities.

Discussion of Results.

It appears from the results obtained on four animal cells and on one plant cell that carbon dioxide can bring about changes in protoplasmic viscosity of two sorts. Clear evidence of liquefaction is obtained in *Paramœcium, Colpidium* and *Spirogyra.* Observations on this point are not available for *Arbacia* eggs, and in the case of *Amœba,* while the results are apparently in agreement with those already mentioned, they are based on mere appearances, and are therefore perhaps not so certain as the others. In every case where liquefaction was shown to occur this change could be shown to be reversible.

As to solidification, the clearest case is furnished by *Paramœcium,* where the process and its complete reversibility up to a certain point can easily be demonstrated. Conditions in *Colpidium* are almost as favorable, except that on account of the greater difficulty in bringing about separation of the food vacuoles in the controls, the increasing viscosity can not be followed as far as in *Paramœcium*. In *Arbacia* eggs it is easy to produce solidification, which in the cases here described was in every case reversible, at least in the physical sense. On account of the complicating effects of artificial parthenogenesis the physiological reversibility is more difficult to demonstrate, but in a number of instances it was shown in an entirely satisfactory manner. In one lot of *Spirogyra* solidification was obtained, but could not be produced in several other lots subsequently studied, and until favorable material is again available it will be impossible to make any definite statements about the reversibility of the process. Finally, in *Amœba*, all of the appearances, at least, of solidification can be produced, though in this animal it is impossible to test the matter by the method of centrifugalization.

Considering in its entirety the evidence which has been given, there seems to be no doubt that carbon dioxide is an efficient agent for changing the consistency of protoplasm reversibly in either direction, the exact effects produced depending on the concentration of the gas, the material experimented upon, the time of exposure and the presence or absence of other dissolved substances in the medium. Since CO_2 has these properties, and since it is so universally present in cells and is produced at different rates at different times, it seems not unlikely that it may prove to be an important factor in bringing about certain of the changes in viscosity that accompany various physiological activities of cells. In the case of dividing eggs the temptation is strong to attempt to correlate the striking variations in the rate of CO_2 production observed by Lyon ('04) with the equally striking changes in protoplasmic viscosity described by Heilbrunn ('20), Chambers ('17) and others. The chief difficulty in this case would appear to be that since carbon dioxide can have either a liquefying or a solidifying effect, the exact nature of the changes produced by it would be difficult to predict in advance.

In the case of amœboid movement also the hypothesis that carbon dioxide may be at least an important agent is an attractive one. As a matter of fact, L. Loeb ('20) and Hyman ('17) have both expressed the idea that some substance is produced as the result of metabolism which causes the necessary changes in protoplasmic consistency. L. Loeb ('20) has further suggested that a change in hydrogen-ion concentration is also concerned. It will be noted that carbon dioxide satisfies both of these requirements. In addition, the evidence presented in the present paper shows that it is capable of producing exactly the sort of changes in protoplasmic consistency demanded by such a theory.

It would be easy to engage in further speculations about the probable rôle of CO_2 in other physiological processes, but such speculations would not be warranted by the present state of our knowledge. It is suggested, however, on account of its universal occurrence in cells, as well as on account of its interesting and in some respects unique physiological peculiarities, that carbon dioxide should at least receive serious consideration in connection with many of the as yet unsolved physiological problems which involve changes in protoplasmic consistency.

Summary.

1. A short exposure to carbon dioxide of various cells causes a decrease, and a longer exposure an increase in the viscosity of the protoplasm.

2. Both of these effects are reversible, though the second one tends to pass into an irreversible coagulation if the exposure is sufficiently long continued.

3. The local application of carbon dioxide to *Amœba* can influence in a striking way the formation of pseudopodia.

4. It is suggested that carbon dioxide may be an important factor in producing many of the natural changes in protoplasmic consistency which have hitherto been unexplained.

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THE VITAL LIMIT OF EXSICCATION OF CERTAIN ANIMALS.

F. G. HALL,

Zoölogical Laboratory, University of Wisconsin.

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Introduction.

Water is one of the most important constituents of living matter. The water content of animals varies from 50 to nearly 100 per cent. of the total body weight. All living matter appears to live in water. Not only is this true of the simplest forms of life, the Protozoa and the Protophyta, which are hardly more than naked protoplasm, but it is also true of the more complex organisms where lymph, blood, and sap are found as liquids; the immediate environment of the individual cell. Any change in the amount of water present may interfere with the activities of the cell. Yet it is a familiar fact to zoölogists that many animals will endure considerable exsiccation.

One hundred and fifty years of experimentation has shown that some animals become immobile when gradually dried out, and are apparently dead. This condition Preyer (1891) called "anabiosis." Leeuwenhoek was perhaps the first to observe this phenomenon in 1701. He found that Rotifera and Tardigrada living in the sand of roof gutters could be completely dried out without losing vitality. Baker (1764) revived nematodes after they had been in a dried state for twenty-seven years. Doyère (1842) found that Tardigrada could endure higher temperatures, as well as extreme exsiccation, without injury and in many cases were able to live even in the absence of oxygen. Semper (1881) tells of experiments with *Apus* and *Cypris* eggs, which he kept in a chest of dried mud for six years. He was able to hatch out larvæ in summer or winter by adding water to the mud. Semper did not believe, however, that the eggs of the many animals that endure extreme exsiccation lose all their water, but that the integument of the egg does not permit the complete removal of water.

Professor W. S. Marshall has kept Trogoderma larvæ alive for more than five years in vials without food or water. Wodsedalek (1921) carried out similar experiments with the same species. The larvæ moulted but became smaller after each moult. They did not eat their moulted skins. In Nature's larger laboratory there are many cases of adaptation of animals to a dry environment. Protozoa and other microscopic forms encyst themselves when ponds dry out. Thus they resist in a motionless state the hot dry winds of summer, but revive again in spring when ponds are filled with water. Spores of bacteria are extremely resistant to exsiccation, living for days on dry glass. Desert lizards live in extremely dry places. In fact, a number of animals live in the driest places on earth.

While there are many experiments to show the effects of exsiccation most of them have been with simpler or smaller animals. Because of the microscopic size of these no quantitative results could be obtained. It would seem improbable that an animal can remain active after losing all its water, or that all the water could be removed from an organism without causing death. Thus it is important to study comparatively the amount of water that can be removed from animals without loss of vitality. Schmidt (1918) found that earthworms can revive and apparently become perfectly normal after a loss of 61.8 per cent. of the body weight, or nearly 73 per cent. of the weight of the water contained in the body. He used the earthworm *Allolobophora foetida* and a desiccator containing calcium chloride. Durig (1901) found that the common European frog would lose 30 to 39 per cent. of the body weight if the drying was slow. Hill (1906) states that if a man loses ten per cent. of his weight in water, he usually dies. Shelford (1913) found in studying the reactions of animals to evaporation that smaller animals died from loss of water much more rapidly than larger. He also states that when the skins of amphibians became dry they did not recover when put into water.

This paper describes the exsiccation of certain animals. Experiments were carried on at the University of Wisconsin under the direction of Professor A. S. Pearse to whom the writer is greatly indebted for many suggestions and criticisms. Professor M. F. Guyer has also given numerous helpful suggestions. Thanks are also due to Dr. H. H. T. Jackson for the identification of the mammals used, and to Drs. J. A. E. Eyster and W. J. Meek for help in the construction of apparatus.

II. Method of Determination of the Vital Limit.

Apparatus.

The apparatus for these experiments consisted of an air-pump, exsiccating jars, filters, and an exsiccating chamber (Fig. 1).

The air-pump was an automatic electric air compressor[1] provided with an air-tank and a pressure gauge. The tank had a capacity of four gallons. A switch controlled the starting and stopping of the motor, whenever the pressure fell to 20 lbs. or rose to 25 lbs. A valve constructed on the air-tank allowed whatever flow of air was deemed necessary. The air-pump was always in the same room with the exsiccating chamber and where only fresh air was pumped and used.

The exsiccating jars (Fig. 1, *P, S*) consisted of four glass jars. The first (Fig. 1 *P*) was a 500 cc. Woulff bottle, full of crushed pumice stone, the pieces ranging from 0.5 mm. to 5 mm. in diameter, and about half full of sulfuric acid C.P. The other three jars were the ordinary washing bottles, filled with sulfuric acid C.P. The four jars were connected in series. Air passing from the compressed air-tank through a trap to prevent back flow of acid was rendered dry by its passage through the exsiccating jars.

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[1] Central Scientific Co. Cat. No. 218, listed as No. 1390.

Two filters were arranged. The first (Fig. 1, *Z*), a 100 cc. bottle containing about an equal weight of powdered and granulated zinc. The purpose of this filter was to prevent the passage of sulfuric acid. The second filter (Fig. 1, *G*), consisting of a 500 cc. bottle filled with glass wool, prevented the passage of any physical impurities. Tests of the air were made by allowing the air as it passed out of the exsiccating chamber to bubble through a methyl-orange solution for an hour. There were no indications of sulfuric acid in the air.

The manometer or flow-meter (Fig. 1, *M*) used for the determination of the rate of flow of air into the exsiccating chamber was constructed of glass tubing. A horizontal tube connecting the two ends of a U-tube and having a small aperture between the points of connection allowed the passage of air from the second filter to the exsiccating chamber. The U-tube was partly filled with olive oil, which was used because of its low evaporating coefficient. The passage of air through the horizontal tube increased the pressure on the column on one side thereby lowering the level. The difference of level of the liquid in the U-tube was a measure of the static pressure and was an indicator of the rate of flow of the air passing into the exsiccating chamber. The manometer was accurately calibrated from a gas meter used for such purposes by the department of physiology, University of Wisconsin. A scale was placed behind the U-tube and the rate of flow in cubic centimeters could be read at any time.

A large Whithall-Tatum museum jar (Fig. 1, *C*) was used for the exsiccating chamber. Four holes were drilled through the lid, one for the dry air inlet, one for the air outlet, two for the inlet and outlet tubes to the dew-point apparatus. The lid was fastened by a metal clamp and sealed by a rubber gasket. An outlet tube connected the exsiccating chamber with a small beaker containing sulfuric acid (Fig. 1, *O*). The end of the outlet tube was just far enough below the surface of the acid to indicate by bubbling if the chamber was tightly sealed, and also to prevent back flow of air containing water.

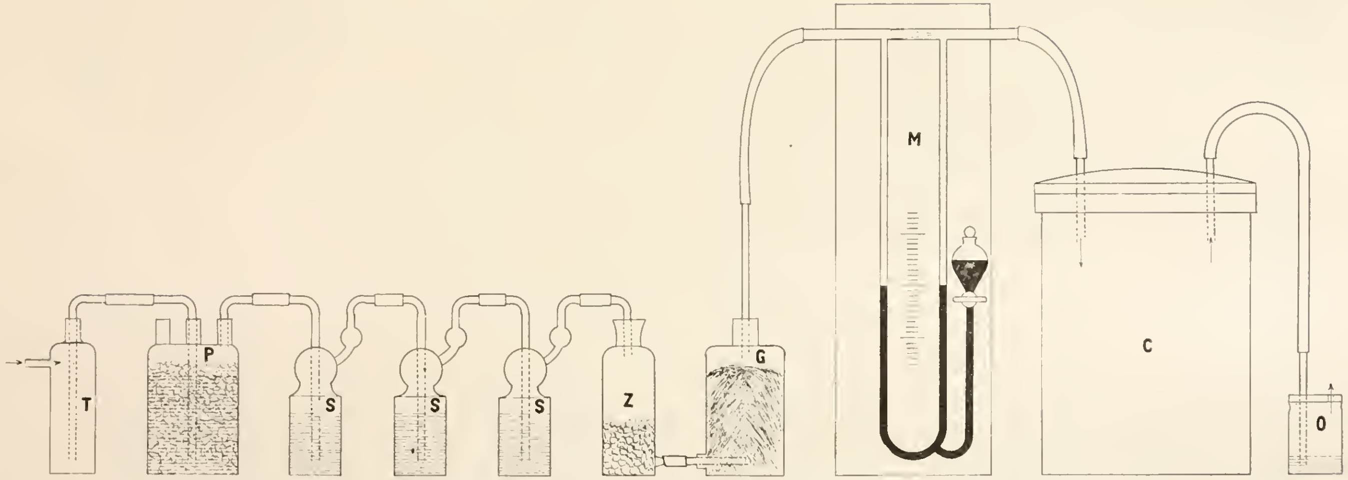

Fig. 1. Plan of apparatus for determining the vital limit of exsiccation of animals. *T*, trap; *P*, pumice stone and sulfuric acid exsiccating jar; *S*, sulfuric acid exsiccating jars; *Z*, zinc filter; *G*, glass wool filter; *M*, manometer; *C*, exsiccating chamber; *O*, outlet.

Plan of Experiments.

The animals used for these experiments, with the exception of the mice, were kept for several days without food; so that their digestive systems were evacuated. They were kept in cages and supplied constantly with water. The mice were kept in cages, and supplied with food and water. All the animals were weighed separately; the earthworms, leeches, and meal worms were weighed and then placed in small flat glass dishes which were covered with cheese cloth. From one to eight dishes were placed in the exsiccating chamber at a time; the salamanders, frogs, snakes, lizards, turtles, and mice were weighed in screen cages and transferred directly to the exsiccating chamber. A screen partition was used to separate the animals when more than one was exsiccated at a time.

The temperature, air-flow in cubic centimeters per minute, and the time were noted on a record card for each individual. The corn or oats fed the mice was weighed and the amount eaten noted each day. The relative humidity was found to vary considerably while animals were in the chamber. With an air-flow of 1,000 cubic centimeters per minute the relative humidity varied from 0 to 10 per cent.[1] The supply of sulfuric acid in the exsiccating jars was renewed whenever there was indication that it had been weakened much by the absorption of water.

After removal from the exsiccating chamber the animals were placed in as normal an environment as could be obtained. The invertebrates were placed on moist filter paper at normal room temperatures. Some individuals were placed in a refrigerator where recovery from exsiccation was more easily accomplished.

The limit of exsiccation was determined by the maximum percentage of water that could be removed from an animal without the loss of vitality. Water was removed from an animal by subjecting it to dry air in an exsiccating chamber (Fig. 1, *C*). The loss of weight was the means used for the determination of the percentage of water lost. Results of these experiments on the vital limit of exsiccation are summarized in Tables I.–XII.

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[1] By means of absorption tubes containing H_2SO_4, c. p. connected at the outlet of the exsiccating chamber (Fig. 1, *C*), not a trace of water was found to pass for a period of 25 hours, providing there was no animal in the exsiccating chamber.

Water Content Determination.

It was thought by the writer to be of interest to ascertain the amount of water normally present in the species that were used in the exsiccation experiments. The water content was determined by drying the animals in a drying oven and weighing at intervals until the weight became constant. The oven was kept at 99° C. The percentage of water in relation to the body weight for several species was as follows: meal worm (*Tenebrio molitor*), 49.8 per cent. (average of 60 individuals); leech (*Placobdella parasitica*), 76.4 per cent. (average of 10 individuals); house mouse (*Mus musculus*), 70.8 per cent. (average of 7 individuals); frog (*Rana pipiens*), 80.8 per cent. (average of 2 individuals). Schmidt (1918) found the water content of the earthworm, *Allolobophora fœtida,* to be 84.1 per cent. (an average of 13 individuals).

III. Experimental Results.

Earthworm.

The species used in this experiment was the *Allolobophora chloroticus* (Savigny). The worms were collected near Lake Mendota during the summer of 1919. They were placed between moist filter paper in flat glass jars and kept for several days before using. They were rolled on dry filter paper in order to remove surface moisture and weighed in small flat dishes before being subjected to exsiccation. Several individuals were exsiccated at a time. Individual worms were removed at intervals. Directly following removal from the exsiccating chamber they were placed on moist filter paper for recovery.

When the worms were exsiccated they became contracted, assumed a dark brown color. and lost all mobility. As exsiccation approached the vital limit the worms lost all elasticity of the body and a mucus-like covering was secreted. It was found that worms after exsiccation, if placed in a refrigerator at a temperature of 10° to 14° C., recovered after a greater loss of water than those that were kept at ordinary room temperature. Perhaps lower temperature retarded bacterial decomposition. Worms Nos. 8–13, and 16–22, inclusive, did not show clitella. Many individuals showing clitella bled considerably. As the clitellum is abundantly supplied with blood vessels, blood effusion probably takes place more readily. Worms also appeared to be killed by infection in some cases.

The results of these experiments, with twenty-two individuals, show: (1) worms were exsiccated up to a loss of 69.6 per cent. of their body weight, or approximately 83 per cent. of the water contained in their bodies, was given up without loss of vitality; (2) lower temperatures were more favorable to the recovery of exsic-

Table I.

Earthworm, *Allolobophora chloroticus* (Savigny).

No.	Weight.		Condition of Exsiccation.			Results.			Condition of Animal after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, c.c./min.	Deg. C.	Time in Hours.	Survived.	Days Lived.	% Exsiccated.	
1	0.473	0.288	500	28	4′ 30″	no	—	61.1	Hard, dry, inactive
2	0.690	0.405	500	28	4′ 30″	no	—	42.7	Hard, dry, inactive
3	0.733	0.328	500	28	4′ 0″	no	—	44.7	Hard, dry, inactive
4	0.626	0.064	600	30	1′ 0″	yes	Indef.[1]	10.2	Active
5	0.570	0.150	600	30	2′ 0″	yes	Indef.	26.3	Active
6	0.223	0.144	600	30	3′ 0″	no	—	64.6	Inactive, dry, hard
7	0.199	0.139	600	30	3′ 0″	no	—	70.0	Inactive, dry, hard
8	1.833	0.870	400	28	2′ 30″	no	—	47.5	Inactive, dry
9	0.220	0.119	400	28	2′ 30″	no	—	54.1	Inactive, dry
10	0.240	0.149	300	28	1′ 30″	yes	2[3]	62.1	Alive, inactive (R)[2]
11	0.273	0.190	300	28	1′ 45″	yes	4	69.6	Inactive, dry (R)
12	0.171	0.091	300	28	1′ 45″	yes	Indef.	54.4	Inactive, dry (R)
13	3.014	0.780	500	27	1′ 45″	yes	1	25.9	Active (R)
14	0.590	0.115	500	27	2′ 0″	yes	Indef.	19.5	Active (R)
15	2.086	0.351	500	27	2′ 40″	no	—	16.8	Inactive, dry (R)
16	2.601	0.443	500	27	3′ 10″	yes	1	17.0	Active (R)
17	0.480	0.150	800	27	2′ 0″	yes	1	31.2	Active (R)
18	0.686	0.224	800	27	2′ 30″	yes	1	32.7	Active (R)
19	0.664	0.195	800	27	3′ 0″	yes	1	29.3	Active (R)
20	0.790	0.204	800	27	3′ 0″	yes	1	25.8	Active (R)
21	0.645	0.281	800	27	4′ 30″	yes	1	42.9	Inactive (R)
22	0.360	0.178	800	27	4′ 30″	yes	1	49.2	Inactive (R)

[1] By "Indef." is meant that the animals lived for forty days or more. The exact number of days was not recorded after the fortieth day.

[2] (R)—revived in refrigerator at 10° to 14° C.

[3] Death accidental.

cated worms; (3) worms not showing clitella dried more slowly and endured more exsiccation than those showing clitella.

Leech.

The species used for these experiments was *Placobdella parasitica* (Say) Moore. Specimens were collected from aquaria containing turtles and methods employed were the same as those used for earthworms with the exception that the leeches were kept under water until used. During exsiccation each leech coiled up into a ball-shaped mass containing air, for when they

TABLE II.

LEECH, *Placobdella parasitica* (Say) Moore.

No.	Weight.		Condition of Exsiccation.			Results.			Remarks.
	Initial, gms.	Lost, gms.	Time. Hours.	Deg. C.	Rate of Air-flow, c.c./min.	Survived.	Days Lived.	% Exsiccated.	
1	0.620	0.323	6′ 15″	22	1,000	yes	6	52.1	R. (13° C.). Hard, dry, inactive.
2	0.865	0.524	6′ 15″	22	1,000	yes	8	60.6	Hard, dry, inactive.
3	0.592	0.403	6′ 0″	20	1,000	yes	5	68.1	Hard, dry, inactive.
4	1.470	0.520	8′ 0″	20	1,000	yes	Indef.	35.3	Dry. active.
5	0.733	0.403	5′ 30″	20	1,000	yes	6	55.0	Hard, dry, inactive.
6	0.542	0.350	8 0″	20	1,000	yes	Indef.	64.6	Hard, dry, inactive.
7	0.430	0.289	5′ 30″	20	1,000	yes	8	67.2	R. (13° C.). Hard, dry, inactive.
8	0.660	0.467	8′ 0″	20	1,000	no	—	70.7	Hard, dry, inactive.
9	0.484	0.341	7′ 30″	22	1,000	yes	17	70.4	R. (13° C.). Hard, dry, inactive.
10	0.566	0.242	5′ 30″	22	1,000	yes	Indef.	57.2	Hard, dry, inactive.
11	0.523	0.349	7′ 30″	22	1,000	no	—	66.7	Hard, dry, inactive.
12	1.175	0.540	9′ 45″	22	1,000	yes	Indef.	46.0	Dry, flexible.
13	0.554	0.370	6′ 15″	22	500	no	—	66.8	Hard, dry. inactive.
14	1.000	0.650	9′ 30″	22	500	no	—	65.0	Hard, dry, inactive.
15	0.476	0.326	9′ 30″	22	500	yes	4	68.7	R. (13° C.). Hard, dry, inactive.
16	0.331	0.256	9′ 30″	22	500	no	—	77.3	Hard, dry, inactive.
17	0.305	0.235	7′ 30″	25	1,000	no	—	77.0	R. (13° C.). Hard, dry, inactive.
18	0.320	0.250	7′ 30″	25	1,000	no	—	78.1	R. (13° C.). Hard, dry, inactive.
19	0.405	0.285	7′ 30″	25	1,000	no	—	70.4	R. (13° C.). Hard, dry, inactive.
20	0.425	0.313	7′ 30″	25	1,000	no	—	73.6	R. (13° C.). Hard, dry, inactive.

were dropped into water after exsiccation small air bubbles arose as the leeches uncoiled. After extreme exsiccation the leeches became hard; the skin dry; all mobility and elasticity were lost; and a mucus secretion enveloped the body, as was the case with the earthworms. During recovery the body, posterior to the twelfth segment, became swollen and was apparently inactive. The anterior part of the body remained active. The posterior part of the body became swollen to twice its natural size and softened. After a few days it broke open and the animal apparently bled to death. Leeches usually showed signs of recovery within an hour after removal from the exsiccation chamber to water.

The results of these experiments, with twenty individuals, show: (1) leeches were exsiccated to 70.4 per cent. of their body weight, or approximately 92 per cent. of the water contained in the body was given up, without loss of vitality; (2) lower temperatures were more favorable to the recovery of the exsiccated leeches.

Meal Worm.

The meal worm, the larva of *Tenebrio molitor* Linn., was used The methods employed were the same as with the earthworm. It was very difficult to tell when the vital limit of exsiccation was reached as the meal worms were inactive for the greater part of the time while in the exsiccating chamber. One meal worm lost a greater percentage of body weight than the average percentage of water contained in the body. The body of the meal worm contains much fat. It is probable that metabolic water was derived from this fat during exsiccation. The body of a meal worm always became considerably compressed during exsiccation.

The results of these experiments, with ten individuals, show: (1) meal worms were exsiccated to 52.6 per cent. of their body weight without loss of vitality; (2) the weight lost was greater than the average weight of water contained in the body; (3) the chitinous integument is probably a factor in offering resistance to evaporation. The meal worms endured exsiccation for a considerable length of time.

TABLE III.

MEAL WORM, *Tenebrio molitor* Linn.

	Weight.		Condition of Exsiccation.			Results.			
No.	Initial.	Lost.	Rate of Air-flow, c.c./min.	Temp. ° C.	Time, Hours.	Survived.	Days Lived.	% Exsiccated.	Condition after Exsiccation.
1	0.223	0	500	22	4	yes	60+	0	Normal.
2	0.245	0	500	22	9	yes	60+	0	Normal.
3	0.190	0.002	500	22	49	yes	60+	1.0	Normal.
4	0.152	0.039	500	22	508	yes	60+	26.3	Inactive.
5	0.190	0.100	500	22	1,084	yes	2+	52.6	Inactive.
6	0.206	—	500	20	1,130	no	—	—	
7	0.195	0.185	500	20	70	yes	60+	5.1	Normal.
8	0.218	—	500	20	1,130	no	—	—	
9	0.190	0.007	500	20	70	yes	60+	3.7	Normal.
10	0.247	—	500	20	1,130	no	—	—	

Amphibia.

Two species of the Amphibia were used: the leopard frog, *Rana pipiens* (Schreber), and the salamander, *Ambystoma punctatum* (Baird). The frogs and salamanders were collected at various times during the year and were kept in a vivarium until used. Two or three specimens were exsiccated at a time, but individual records were kept. After extreme exsiccation neither frogs nor salamanders showed signs of life. There was not a reflex action to indicate that they were alive, though the temperature was 20° to 22°C. After they had been placed in water, following removal from the exsiccation chamber, during an hour they had always revived enough to swim. During one experiment weights were taken of a salamander to show how soon the weight lost could be regained. Within 24 hours the animal had regained 97.7 per cent. of the weight lost by the body during exsiccation.

The results of the experiments with salamanders, with nine individuals, show: (1) salamanders were exsiccated to 47 per cent. of their body weight without loss of vitality; (2) salamanders gave up water easily and regained water quickly when returned to their natural environment.

The results of the experiments with frogs, with six individuals, show: (1) frogs were exsiccated to 41.0 per cent. of their body weight, and that 50 per cent. of the water contained in their bodies was given up without loss of vitality.

TABLE IV.

SALAMANDER, *Ambystoma punctatum* Baird.

	Weight.		Condition of Exsiccation.			Results.			
No.	Initial, gms.	Lost, gms.	Deg. C.	Rate of Air-flow, c.c./min.	Time, Hours.	Survived.	Days Lived.	% Exsiccated.	Condition and Behavior after Exsiccation.
1	27.030	4.230	20	1,200	9′ 0″	yes	Indef.	15.6	Normally active.
2	37.500	4.300	18	1,000	13′ 30″	yes	Indef.	11.5	Normally active.
3	44.620	4.520	14–17	600	23′ 0″	yes	Indef.	10.1	Normally active.
4	38.600	4.800	14–17	600	23′ 0″	yes	Indef.	12.4	Normally active.
5	36.600	13.760	22	800	51′ 0″	yes	Indef.	37.6	Active, dried up appearance.
6	44.050	10.580	22	800	31′ 0″	yes	Indef.	24.0	Normally active.
7	23.370	11.170	8–20	500	188′ 0″	no	—	47.9	Dead, hard, dry.
8	35.550	15.750	8–20	500	144′ 0″	yes	Indef.	44.4	Inactive, dry, no signs of life.
9	38.450	18.070	20	600	116′ 0″	yes	Indef.	47.0	Inactive, dry, no signs of life.

TABLE V.

FROG, *Rana pipiens* Schreber.

	Weight.		Condition of Exsiccation.			Results.			
No.	Initial, gms.	Lost, gms.	Rate of Air-flow, c.c./min.	Deg. C.	Time, Hrs.	Survived.	Days Lived	% Exsiccated.	Condition after Exsiccation.
1	38.100	4.300	400	26	7	no	—	11.3	Inactive, dead.
2	37.000	3.900	400	26	7	yes	20 +	10.5	Active.
3	22.800	9.340	1,000	22	33	yes	30 +	41.0	Skin dry, apparently dead.
4	22.305	9.795	1,000	21	32	no	—	43.5	Inactive.
5	16.460	5.930	1,000	20	24	yes	30 +	36.0	Apparently dead, skin dry.
6	18.230	8.270	1,000	21	31	no	—	45.6	Inactive.

Reptilia.

Five reptiles were used: the garter snake, *Thannophis radix* (Baird); the painted turtle, *Chrysemys marginata* Agassiz; the American chameleon, *Anolis carolinensis* Cuvier; the horned "toad," *Phrynosoma cornutum* Harlan; the desert lizard, *Sceloporus spinosus floridanus* Wiegmann. Only one snake was exsiccated and it died before removal from the exsiccation chamber.

The turtles were collected from the Madison lakes and were kept in a vivarium until used. As the shell of a turtle adds considerable weight to the body, the water content is not as high, and exsiccation cannot be carried as far. The turtles were revived from exsiccation by removal to an aquarium. Turtle No. 1 lost all activity after exsiccation. Its eyes were sunken, and the skin became very dry. The turtle appeared to be dead, but when cut open its heart was still beating.

The chameleons, horned "toads," and desert lizards were procured from a collector in Texas. The same method of exsiccation was employed as in the preceding experiments. No food was given. The chameleons were exsiccated at a comparatively rapid rate. Their skin became shrivelled, and eyes sunken. The exsiccation of the horned "toads" and desert lizards was very slow. The dry air apparently had no injurious effect upon them until a few days previous to death. At that time they gasped

TABLE VI.

TURTLE, *Chrysemys marginata* Agassiz.

No.	Weight.		Condition of Exsiccation.			Results.			Condition after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, $\frac{\text{c.c.}}{\text{min.}}$	Deg. C.	Time, Hrs.	Survived.	Days Lived.	% Exsiccated.	
1	347.500	114.960	700	20	288	yes	1	33.1	Heart beating normally.
2	281.500	94.000	1,000	20	257	no	—	33.3	Inactive, dead.
3	334.000	94.800	1,000	20	304	yes	Indef.	28.4	Inactive, few signs of life.

continually and breathing seemed forced. As no food was given them, starvation was probably a factor in producing death. It is very remarkable that these animals can endure exsiccation for so long a time without food or water, in perfectly dry air. They lived for nearly four months, apparently with no discomfort. It is easy to understand how they can live in desert areas where many other animals would perish.

TABLE VII.

CHAMELEON, *Anolis carolinensis* Cuvier.

No.	Weight.		Condition of Exsiccation.			Results.			Condition after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, c.c./min.	Temp. ° C.	Time, Hours.	Survived.	Days Lived.	% Exsiccated.	
1	2.110	0.205	1,000	20	17	no	—	10.8	
2	2.360	0.880	1,000	20	184	yes	40+	28.8	Active.
3	1.910	0.885	1,000	20	186	yes	40+	46.3	Active.
4	1.315	—	1,000	20	48	no	—	—	
5	2.190	0.837	1,000	20	168	no	—	36.9	
6	1.220	0.350	1,000	20	195	yes	40+	28.8	Active.

TABLE VIII.

DESERT LIZARDS, *Phrynosoma cornutum* and *Sceloporus spinosus*.

Species.	No.	Weight.		Condition of Exsiccation.			% Exsiccated.	Survived.
		Initial.	Lost.	Rate of Air-flow, c.c./min.	Temp., ° C.	Time in Days.		
P. cornutum ..	1	31.510	10.640	800	21	119	33.8	no
" " ..	2	55.850	10.680	800	21	114	19.1	no
" " ..	3	41.250	—	800	21	22	—	no
S. spinosus...	1	38.300	11.340	800	21	86	29.6	no
" " ...	2	9.400	4.500	800	21	62	47.8	no

The results of these experiments show: (1) Turtles were exsiccated to 33.1 per cent. of their body weight without loss of

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[1] This specimen was killed after exsiccation for an examination of the anal bladder.

vitality; and chameleons were exsiccated to 46.3 per cent. of their body weight without loss of vitality. (2) Horned "toads" and desert lizards were exsiccated to 33.8 per cent. and 47.8 per cent. of their body weight, respectively, before death occurred. (3) Horned "toads" and desert lizards endured exsiccation and starvation for 119 and 86 days, respectively, before death occurred. (4) Reptiles are apparently better fitted to resist exsiccation than any other group of animals experimented upon.

Mammalia.

As representatives of the mammals, three rodents were used: the house mouse, *Mus musculus* (Linn.); the wood mouse, *Peromyscus leucopus noveboracensis* (Fischer); and the meadow mouse, *Microtus pennsylvanicus* (Ord.). The mice were caught in traps and kept in cages in a vivarium with food and water before them constantly. During exsiccation corn and oats were fed and the amount eaten determined by subsequent weighing. Mice remained active until the vital limit of exsiccation was apparently reached and then died quickly if not removed to a suitable environment. The mice became very nervous after subjection to the dry air for a few days but became drowsy as the vital limit was approached.

TABLE IX.

WOOD MOUSE, *Peromyscus leucopus noveboracensis* (Fischer).

No.	Weight.		Condition of Exsiccation.			Food.	Results.			Condition of Animal after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, $\frac{\text{c.c.}}{\text{min.}}$	Temp. ° C.	Time in Hours.	Gms. Oats; Corn.	Survived.	Days Lived.	% Exsiccated.	
1	14.120	4.900	1,000	20	144	10	no	—	34.7	Dead.
2	15.630	—	1,000	20	118	10	no	—	—	Dead.
3	11.790	3.440	1,000	20	75	3	yes	Indef.	29.2	Active.
4	12.230	3.760	1,000	21	77	20	yes	Indef.	30.7	Active.

The results of the experiments with mice, summarized in Tables IX.–XI., show: (1) The house mouse was exsiccated to

24.2 per cent. of its body weight, or about 34 per cent. of the water contained in its body was given up, without loss of vitality. (2) The wood mouse was exsiccated to 30.7 per cent. of its body

TABLE X.

MEADOW MOUSE, *Microtus pennsylvanicus* (Ord.).

No.	Weight.		Condition of Exsiccation.			Food.	Results.			Condition of Animal after Exsiccation.
	Initial.	Lost.	Rate of Air-flow, c.c./min.	Temp. ° C.	Time in Hours.	Gms. Oats; Corn.	Survived.	Days cated.	% Exsic- Lived.	
1	32.110	9.930	1,000	20	48	2	yes	2	30.8	Active.
2	21.780	10.450	1,000	20	54	2	yes	1	48.0	Slightly active.
3	31.460	7.580	700	19	48	3	yes	Indef.	24.1	Active.
4	28.830	9.450	1,000	20	68	3	yes	Indef.	32.1	Active.

TABLE XI.

HOUSE MOUSE, *Mus musculus* Linn.

No.	Weight.		Condition of Exsiccation.			Food.	Results.			Behavior, Etc.
	Initial, gms.	Lost, gms.	Time, Hrs.	Deg. C.	Rate of Air-flow, c.c./min.	Gms. Corn; Oats.	Survived.	Days Lived.	% Exsiccated.	
1	9.530	1.140	12	20	500	0	yes	Indef.	12	Normal.
2	13.820	3.200	65	20	600	0	[1]	[1]	23.3	
3	16.540	3.980	263	20	1,000	60	[1]	[1]	24.1	
4	14.860	3.460	308	20	1,000	25	[1]	[1]	23.3	
5	14.300	1.900	383	20	500	30	yes	Indef.	13.3	Inert, gasping for breath, at removal.
6	16.320	4.590	145	20	1,000	15	yes	—	28.1	Active.
6	16.320	5.735	191	20	1,000	15	no	—	35.1	Dead.
7	20.050	2.930	72	21	1,000	20	yes	Indef.	14.6	Active.
8	15.230	3.780	270	21	1,000	40	yes	Indef.	24.2	Active.

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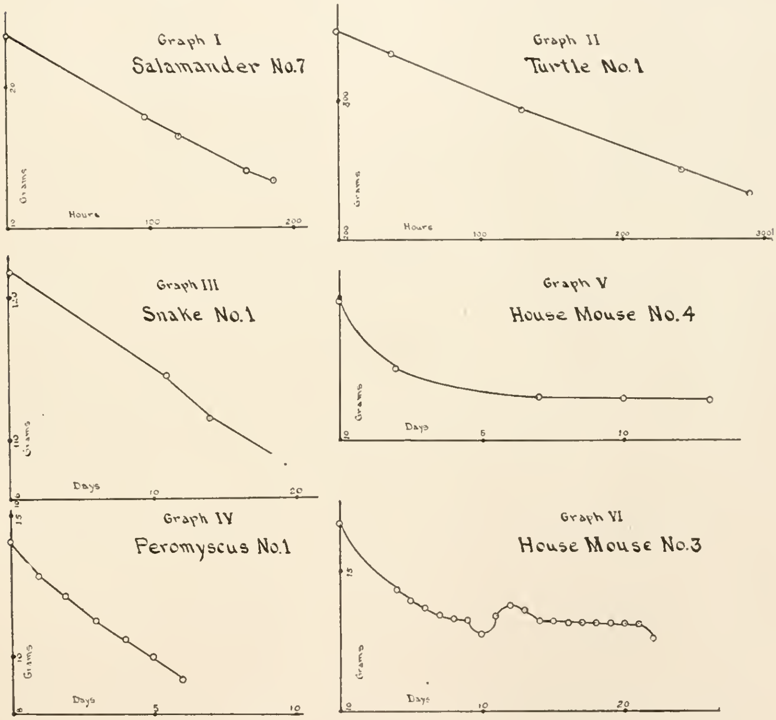
[1] These mice were dead when removed and the weight recorded is the last weight made before they died. As the exact time of death was not known, no weights of the dead mice were taken. The last weight was taken in each case from 12 to 24 hours before death.

weight without loss of vitality. (3) The meadow mouse was exsiccated to 32.1 per cent. of its body weight without loss of vitality.

Graphs.

The graphs shown in Fig. 2 represent the loss of water during exsiccation for intervals of time. It will be noted that in the graphs, with the exception of the house mouse, the water lost for intervals of time is constant. In the case of the house mouse, however (graphs V., VI.), the downward curve indicates a rapid loss of water for the first few days, it then straightens and is nearly horizontal until death of the mouse occurs.[1] These facts indicate a resistance in the house mouse to evaporation—a resist-



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[1] The irregularities in Graph VI. for the ninth to the twelfth days are due to the absence of food the ninth day and the excess eaten the following day.

ance that inhibits evaporation beyond a certain limit. Obviously, a mouse got some water from the corn eaten. The corn was tested and was found to contain less than eight per cent. water. Thus the amount of water obtained from the small amount of corn eaten would be too small to supply the daily loss. Probably metabolic water plays a part in replacing that evaporated.

Discussion.

The chief functions of water in animals are: (1) to dissolve nutrients, (2) to serve as a medium for their distribution, (3) to aid excretion by removing waste products from cells, and (4) to control the body temperature. Water is the most abundant constituent of the living organism and is essential to all life. The relative amount of water contained in animals depends upon the kind of organism, the period of growth, and the nature of food, environment and activities.

In these experiments an attempt has been made to determine the effect of a dry environment upon the amount of water present in organisms, and the effect of exsiccation upon the vital activities. There is need of more complete knowledge of the effects of exsiccation and though the present paper does not settle all problems relating to the loss of water by animals, it adds certain facts that may be of general interest. The different types of animals were subjected to comparable conditions.

It is apparent that the water content of different species varies greatly. The relative amount of water that may be lost and the relative length of time that animals can endure exsiccation also varies considerably. The important factors which limit such variations are: the kind of animal, the size of the animal, the integument, the general metabolism, and the elimination of body wastes.

The results of the writer's experiments apparently support the statements of Babcock (1912), that the need for water is much less for animals that excrete uric acid than those that excrete urea, since uric acid, being practically insoluble in the body fluids, is not as poisonous as urea, and is excreted in solid form with the loss of very little water. Weese (1912) has shown that the excretions of horned "toads" are largely uric acid. This is probably true for many insects.

The migration of the amphibians from water to land is regarded as a very important event in the evolution of animals. Closely associated with the adoption of a terrestial mode of existence by the amphibians is a change in the water content (Oswald. 1917). Before the frog takes to land, for instance, it consists of ninety-three per cent. water. When it becomes a land animal much of its water is lost. During its terrestial life a frog lives in moist places and when subjected to extreme dryness loses much of its water. In the writer's experiments as much as forty-one per cent. of the body weight, or fifty per cent. of the water contained in the body, was given up without loss of vitality. As water is given up so readily, areas where the evaporation rate is high would be generally unfavorable for frogs and for amphibians in general. Shelford (1913) has shown that amphibians, and other animals as well, react to evaporation gradients and concludes that the evaporating power of the air is probably the best index of the suitability of conditions for land animals.

Water does not evaporate from animals as it does from a surface of free water. Even those animals in which the integument permits little apparent evaporation, are able in some way to retard evaporation of water. To test this point a small stender dish containing 30 cc. of distilled water was subjected to the same conditions as a salamander during exsiccation. The area of surface exposed including skin, mouth, and lungs of a salamander weighing thirty-five grams, was estimated to be 121 square centimeters. The average amount of water lost from the dish having an area of 22.9 square centimeters was 4.520 grams per day. The average loss from salamanders was 0.770 grams per day. Of the animals used in the experiments described in this paper the salamander showed the most rapid rate of evaporation, yet the rate of evaporation from an exposed surface of free water was thirty times as great.

General Summary.

1. Animals in general are very susceptible to loss of water in atmospheres of low relative humidity.

2. Animals may lose a very large percentage of the water contained in their bodies without loss of vitality.

3. The amount of water that can be lost by animals without fatal results is in general greater in the less complex animals.

4. The length of time that animals can endure atmospheres of low relative humidity in general depends primarily upon the kind of integument and secondarily upon the proportion of the body surface to the body mass.

5. Metabolism of an organism is an important factor in its resistance to exsiccation.

Table XII.

Comparative Results of Exsiccation Experiments.

Species.	Table.	Per Cent. Exsiccated of		Time Required.
		Body Weight.	Water Content.	
Allolobophora chloroticus	I.	69.6	83	105 mins.
Placobdella parasitica	II.	70.3	92	450 mins.
Tenebrio molitor	III.	52.6	105	1,084 hours
Ambystoma punctatum	IV.	47.0	—	116 hours
Rana pipiens	V.	41.0	50	33 hours
Chrysemys marginata	VI.	33.1	—	288 hours
Anolis carolinensis	VII.	46.3	—	186 hours
Phrynosoma cornutum	VIII.	33.8	—	119 days
Sceloporus spinosus	VIII.	47.8	—	86 days
Peromyscus leucopus	IX.	30.7	—	77 hours
Microtus pennsylvanicus	X.	32.1	—	68 hours
Mus musculus	XI.	24.2	34	270 hours

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Vol. XLII. February, 1922. No. 2

BIOLOGICAL BULLETIN

STUDIES ON INSECT SPERMATOGENESIS. III. ON THE STRUCTURE OF THE NEBENKERN IN THE INSECT SPERMATID AND THE ORIGIN OF NEBENKERN PATTERNS.

ROBERT H. BOWEN.

(From the Department of Zoölogy, Columbia University.)

INTRODUCTION.

In the preceding "Study" of this series I have given a rather full account of the formation of the hemipteran spermatid together with a history of its various components in the subsequent transformation into the mature sperm. An outline of the behavior of the mitochondria (nebenkern) was included in that account, but it was thought best to postpone any extended consideration of details to a later paper. It is the purpose of this paper to complete the description of the nebenkern especially with respect to the complicated and fantastic "patterns" which are its chief characteristic in the insect spermatid.

By the term "pattern," I refer to those curious appearances, which have been likened to a blackberry, an onion, a ball of twine, a skein of yarn, a spireme, and other similar objects, which develop in the nebenkern of the early spermatids and eventually disappear again. It is rather disconcerting to find that after thirty-five years of observation these appearances are not yet understood—have never, indeed, been carefully examined as to origin and significance except by Gatenby, whose recent work on the Lepidoptera has served me as a point of departure for this paper. I have not been able to find with certainty who first noted these patterns—the early workers seem to have taken them as a matter of course and to have given them no particular emphasis.

The earliest clear account which I have discovered is that of von la Valette St. George ('86*a*), who clearly figured the "onion" stage in *Blatta* (see his Fig. 74), and likened it to a ball of thread. Later, he described much the same appearance in *Phratora* ('86*b*) and (doubtfully) in *Forficula* ('87). These accounts were the forerunner of a long series of similar observations in which the distinctive feature of nebenkern structure is a more or less thread-like appearance, or more correctly, a division of its substance into layers which are often likened to those of a hemi-sected *onion*. Another characteristic appearance, occurring often times in conjunction with the preceding, was first described by Platner ('89), who noted the differentiation of the nebenkern in Lepidoptera into two substances concentrically arranged—an inner, darkly stained core enclosed in an outer clear, non-staining material. Subsequent observers have found in many forms a similar peripheral zone, often broken up into vacuoles by thin partitions traversing the clear substance. Still a third type of pattern has been described particularly by Holmgren ('02) in *Silpha,* and by Vejdovský ('12) in *Diestrammena,* consisting of a series of smooth or beaded cords running lengthwise through the nebenkern as it begins its elongation. This appearance seems to have been seen by comparatively few observers, and to have been misinterpreted by all. The problem is, therefore, a twofold one: (1) what is the structural basis of these patterns? and (2) what is the relation of these various appearances to each other and to the course of sperm formation as a whole? In the account which follows, I have tried to give at least a descriptive answer to these questions.

The material for this "Study," as in the preceding ones, has been drawn from Hemiptera belonging to the Family *Pentatomidæ.* As I have already pointed out (Bowen, '20), this particular family is characterized by the occurrence of numerous species in which certain of the spermatocytes and spermatids are unusually large as compared with the remaining ones, which are strikingly smaller ("normal") in size. The occurrence of these *large* spermatids, with *correspondingly large nebenkerns,* has made possible a structural analysis of the nebenkern which would have been exceedingly difficult, if not entirely impossible, in the spermatids of "normal" size. This paper, therefore, deals exclusively with the large-cell generations, but the results have been checked in the small-cell generations and it is clear that the phenomena are in both cases essentially the same, the factor of size being the sole difference. I have used for this study three forms—*Euschistus euschistoides* Voll., *Brochymena quadripustulata* Fab., and *Murgantia histrionica* Hahn, each of which possesses two testicular lobes of large-cell generations.

I have made use particularly of three technical methods—Benda's alizarin-crystal violet stain and the Golgi methods of Kopsch and Cajal, the reasons for employing these particular methods being made evident by the descriptive section which follows. The first named was used exactly as described in the second of these "Studies." The Kopsch method does not usually impregnate the mitochondria, but occasionally the nebenkern and its derivatives are blackened—in the present case after an immersion in 2 per cent. osmic acid of about ten days. The Cajal formol-uranium nitrate method was used as described by Cajal,[1] with a primary fixation of 8 to 10 hours, followed by silver nitrate (1.5 per cent.) for 36 or 47 hours, and reduction with hydroquinone for periods up to 24 hours. At best this method is a rather capricious one.

I have here to acknowledge the many helpful suggestions and criticisms which have been contributed from time to time by Professor E. B. Wilson. I am also indebted to Dr. Franz Schrader for assistance in the collection of material, and particularly to Mr. H. G. Barber through whose unusual kindness I was able to get the specimens of *Brochymena* which I have used in this "Study." Mr. Barber has also identified all of my material.

Observations and Discussion.

In an earlier work (Bowen, '22), I have given a detailed account of the manner in which the spermatid nebenkern is condensed from the originally thread-like (with some inter-mixed granules) mitochondria into a compact, spherical chondriosome body. In the large spermatids this body usually appears, immediately after formation, of rather homogeneous texture, staining faintly or not at all with crystal violet. In the small spermatids the nebenkern typically stains an intense violet (after Benda). Thus, if there is any structural differentiation of the nebenkern in this initial stage it is lost in one case by failure to stain and in the other by over-staining. Subsequent phenomena indicate, however, that there must be at this time some differentiation of the mitochondrial substance, and it will first be necessary to examine carefully the evidence bearing on this point.

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[1] See Cajal, Algunas variaciones fisiológicas y patológicas del aparato reticular de Golgi., Trab. Lab. Biol. Univ. Madrid, 1914, Vol. 12.

As other observers have remarked, the mitochondria in the spermatocytes at the time of the maturation divisions appear characteristically in the form either of granules or of rods and threads of various dimensions. As Meves ('00) was the first to show (in *Pygæra*), the granular mitochondria are not homogeneous spheres, but are actually vesicles the substance of which is differentiated into two distinct parts—an intensely staining envelope or peripheral layer and a clear, non-staining interior. This structure is very clearly demonstrated in many other Lepidoptera, as recently shown by Gatenby ('17), who has applied the convenient descriptive adjective *chromophilic* and *chromophobe* (or better, *chromophobic*), to the staining and non-staining portions respectively of the chondriosome vesicles. The same vesicular structure has also been shown to occur in some forms other than insects (*e.g.,* Mollusca), so that it would appear reasonable to expect a similar duplex structure in mitochondria of the rod type. The evidence here is not entirely convincing, due largely to the difficulties of technique. Meves ('07) has, however, noted that in the thread-like mitochondria of the honey-bee, there is a differentiation into an outer, chromophilic envelope with a core of non-staining material. Gatenby ('18) has recently stated that in the beetle (*Tenebrio*) the mitochondria occur either as "spheres" or as "elongated tubes, containing internally a chromophobic substance." In the Hemiptera, I have never been able to satisfy myself of this duplex structure. The mitochondria are in the form of long, delicate threads (Bowen, '20) which are so fine that it has not yet been possible to distinguish any internal structural features. However, in one case, *Murgantia histrionica,* I have found that the mitochondria at an early spermatocyte period pass through a granular stage in which it is possible clearly to make out the same differentiation into two substances which others have noted in the like granular mitochondria of Lepidoptera. Subsequently the granules produce the long, thread-like mitochondria typical of *Euschistus* and other pentatomids. It is, I think, reasonable to presume that the structure of the granules is carried over into the threads, which would thus correspond in structure with those of *Tenebrio.* Without going further into these purely structural aspects, it seems to me fair to conclude that in the male germ cells at least the mitochondria are generally composed of two distinct substances, related to each other as I have described above. Whether there is any relation between this morphological duplicity and the presumed chemical composition (lipoid plus albumenoid) of the mitochondria is entirely problematical. Indeed, it is not unlikely that our ideas of the chemical nature of these bodies will have to be revised in the light of recent cytological investigations.

Assuming, then, a duplex morphological structure for the mitochondria, we are now in position to take up the structure of the nebenkern at the time when it has just rounded out into its typical shape. Here, again, our problem is simplified by a preliminary study of the formation of a nebenkern from the granular type of mitochondria. This was first observed in detail by Meves ('00) in *Pygæra,* and more recently the same ground has been worked over by Gatenby ('17) in a most illuminating manner. Meves showed that at the close of the second maturation division, the vesicular mitochondria begin to fuse together into an irregular, darkly staining mass containing many large, non-staining vacuoles. Subsequently, the stained substance is aggregated into a roughly spherical mass enclosed in a non-staining envelope which is very sharply delimited externally and is traversed by numerous strands passing out from the central, chromophilic core. These latter disappear eventually, and the nebenkern is smoothed out into the two zones observed many years before by

Platner ('89). Meves concluded that in the process of condensation, the constituent materials of the original mitochondrial vesicles had simply exchanged places, the chromophilic substance having run together to form a homogeneous, central core, for which the formerly internal, chromophobic substance now acts as an envelope.

Gatenby's ('17) account[1] is much more complete and proves conclusively that the details as given by Meves are badly distorted through the use of acetic acid in the fixative. Gatenby shows that the running together of the mitochondrial vesicles occurs in a rather orderly way, the chromophobic material gradually fusing to form the main body of the nebenkern within which the chromophilic substance is molded into a long, but perfect spireme wound up, as it were, within the nebenkern proper. Omitting, for the present, any discussion of details, it is clear that the nebenkern thus formed is not homogeneous, but quite the contrary, the exact (apparent) pattern assumed being dependent on the perfection of the technical treatment. The essential result has been the fusion of the chromophobic material of the various chondriosome spheres to form a continuous, homogeneous matrix in which is embedded the chromophilic material at first in a fantastic, thread-pattern. How is this result to be compared with the nebenkern formed by the fusion of thread-like mitochondria?

Since it has not been possible to differentiate the chromophobic material in the thread-like mitochondria of the Hemiptera, one can not trace the gradual evolution of the nebenkern as Gatenby has done; although in preparations not too darkly stained one sometimes gets the impression that the substance producing the nebenkern is not as homogeneous as it often appears to be. Once fully completed (perhaps even earlier), the nebenkern begins to show the first clear signs of a differentiation into two substances. This first makes itself evident by the appearance of a large number of small vacuoles in the periphery of the mitochondrial mass (Fig. 1). These are at first very indistinct and difficult to demonstrate, but they presently become clearer and form a complete layer investing the periphery of the nebenkern. They seem to stain little or not at all, in contradistinction to the central mass which is more darkly colored. This may be due to the fact that the process of differentiation is going on from without inwards, or it may be due to the optical superposition of several layers of vacuoles. I am rather inclined to think that the process of differentiation is going on throughout the whole mass, but that the peripheral portions perhaps progress more rapidly. It is a curious fact that in many Benda preparations which seem to be otherwise excellent, these and the later differentiation phenomena are not demonstrated at all. Further, I have not been able to get any preparations (of *Brochymena*) stained with sufficient sharpness to show these vacuoles as they should, of course, appear on the rounded surfaces of the nebenkern as one focuses up and down. Zweiger ('07), however, seems to have made out this stage in *Forficula* (see his Fig. 36), in which the whole nebenkern seems made up of very small vacuoles.

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[1] So far as I know, Gatenby's results have not yet received any confirmation from other workers. It is, therefore, possible that with further study of the nebenkern in Lepidoptera other interpretations of the "spireme" structure as described by Gatenby will be suggested. However, for the purposes of this discussion, I have considered Gatenby's account as correct, and the conclusions reached will not be affected in any important way should the details of the "spireme" formation fail of substantiation.

The process of differentiation by which these vacuoles are produced has doubtless been proceeding for some time, but is made visible only when the vacuoles attain dimensions within the reach of our technique. At all events, Fig. 1 represents only a transitory condition in a continuous process which is progressing toward a definite end. The successive figures which I have drawn are merely selected at convenient points along the way. Fig. 2 represents a stage somewhat later than the preceding figure, in which the nature of this vacuole formation has become much more evident. It is clear now that the formation of non-staining vacuoles is going on throughout the nebenkern mass, the outer ones apparently being differentiated more rapidly than the more interior ones. Thus it comes about that these vacuoles are arranged in rather definite layers which, in cross-section, would appear as concentric rings (Fig. 2).[1] The outermost layer clears up very rapidly, the separate vacuoles fusing together to form large clear spaces separated from each other by septa which pass outward from the central mass to the exterior of the nebenkern, which is marked by a very definite membrane. The whole thing rather recalls the figure given by Meves ('00) of *Pygæra,* though no real comparison is, of course, possible.

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[1] This corresponds approximately to the period at which the centriole migrates from its originally anterior position (Fig. 1) to its definitive position at the base of the sperm head (Fig. 3). (See Bowen, '22.)

It is probably the stages between Figs. 1 and 2 that have often been noted heretofore and likened to a "blackberry." It is clear that if, as often happens in the small-cell generations, the nebenkern as a whole took the stain rather heavily, its appearance would be something like that of a spherical mass of soap-bubbles —or a blackberry. The many bizarre figures of this condition would seem to indicate that fixation effects may play a rôle in determining the exact appearance of the final result. That the main features of this stage are real, is indicated, however, by its many reproductions in the literature of insect spermatogenesis. Thus, Henking's ('91) Fig. 63 and Paulmier's ('99) Fig. 43 are excellent reproductions of the "blackberry" stage in *Pyrrhocoris* and *Anasa* respectively, very similar to those which I have seen in *Murgantia.* The early stages in the development of the vesicles as seen in optical cross-section are clearly figured by Voinov ('03) in *Cybister* (Fig. 50), by Zweiger ('07) in *Forficula* (Fig. 37), and by Shaffer ('17) in *Passalus* (Fig. 19). Later stages in the condensation of the peripheral layer of vacuoles are figured by Holmgren ('02) in *Silpha* (Fig. 9*f*), by Gross ('07) in *Pyrrhocoris* (Fig. 98) by Stevens ('05) in *Blatella* (Fig. 150), and (probably) by Doncaster and Cannon ('20) in *Pediculus* (Figs. 20 and 21). However, it is not improbable that some of these cases are artifacts similar to those figured by Meves ('00) in *Pygæra* as mentioned above.

The clearing up of the outer layer of vacuoles now proceeds rapidly, the substance of the vacuole walls being apparently withdrawn into the underlying region of the nebenkern, and eventually the whole peripheral zone appears as a clear, non-staining envelope enclosing a central, chromophilic core (Fig. 3). Usually a connection or two is still retained between the external membrane of the nebenkern and the central portion, these connections being apparently indeterminate in position. At the same time it becomes evident that the vacuolated condition of the central mass is likewise clearing up, the substance of the vacuole walls being condensed into concentric shells of chromophilic material marking the limits between the former concentric rings of vacuoles, while the substance of the vacuoles themselves flows together to form more or less continuous shells of non-staining material alternating with the thinner shells of chromophilic material. This arrangement is at first very incomplete and imperfect (Fig. 3), the chromophilic partitions being more or less irregular. Something of this intermediate period in the differentiation of the nebenkern is also figured by other workers, particularly by Wilke ('07 and '13) in *Hydrometra*. (See his Fig. 70 in *Hydrometra lacustris* ('07) and especially Figs. 77 and 74 in *Hydrometra paludum* ('13).)

At the period of Fig. 3, the shells of chromophilic substance are still joined at many irregular intervals by cross partitions. Presently, however, the pattern clears up considerably and becomes much more regular in its main outlines (Fig. 5). The concentric zones of material are now smoother and more nearly complete, and the nebenkern as a whole exhibits a tendency to a division of all the parts into two equal halves (Fig. 5). The general arrangement recalls the appearance of a hemi-sected "onion" —noted by so many students of insect spermiogenesis. Previous to this time the pattern seems to have been susceptible to the disintegrative action of acetic acid, but with the stronger development of the chromophilic plate-work, the pattern is very often preserved after almost any fixative. This stage has thus become the most familiar of the nebenkern "patterns." It was figured by von la Valette St. George ('86*a*) in *Blatta* (Fig. 74) and later described in detail by Henking ('91) in *Pyrrhocoris*. Wilke in *Hydrometra lacustris* ('07) (Figs. 72 and 75), and in *H. paludum* ('13) (Fig. 78) has figured this stage with what one is inclined to think a trifle too diagrammatic clearness. Attention may also be called to the figures by Stevens ('05) of *Stenopelmatus* (Figs. 84 and 85) and *Blatella* (Fig. 151), by Wassilieff ('07) of *Blatta* (Fig. 56), and by Boring ('07) of *Pœciloptera*

(Fig. 278). To other workers this stage has looked like a threadwork of some sort, an appearance due possibly to the disturbances of fixation or the failure to stain clearly (but compare also with Gatenby's description). Thus, compare Henneguy ('96) on the "filamentous" nebenkern in *Pyrrhocoris,* and other early workers. Many observers have also noted the curious disposition of the "layers" of the nebenkern to take the stain differently. This is particularly common after the osmic acid fixatives (Flemming), the results being often very fantastic and unexpected. Gross ('07) has figured a variety of such results in *Pyrrhocoris,* and I have seen much the same thing in the pentatomids. The most interesting of these phenomena is that of alternative staining, in which the different layers on one side of the mid-line are stained in various degrees while in the other half of the nebenkern the degrees of staining are exactly reversed. (See Gross ('07) Figs. 100 151, 154, 155, and 156.) The meaning of these peculiar phenomena is entirely unknown.

The structural foundation of these patterns will be made somewhat clearer by a comparison of the nebenkern as seen from the side (Fig. 5) and from one pole (*i.e.,* in cross-section) (Fig. 6). It is evident, in the first place, that the substance of the nebenkern has become sharply differentiated into two substances of very different staining capacities. One of these stains little, or not at all, and forms a more or less homogeneous matrix in which is embedded a mass of material that takes the usual mitochondrial stains (crystal violet, for example). Further, this stained substance is arranged in very characteristic concentric rings, the successive rings being separated by zones of the non-staining matrix. These rings might possibly be taken for a tangled threadwork comparable to the "spireme" of Gatenby ('17), but by focusing up and down it is clearly proved that these rings are not threads at all but more or less extensive, shell-like plates. This is made even more clear by Fig. 6 in which the nebenkern is cut at right angles to that of Fig. 5. It is quite obvious that the apparently concentric rings are in reality the optical cross-sections of a more or less complete plate-work, of which the imperfectly spherical plates are arranged one within the other in a concentric series. *The patterns are merely optical cross-sections of a plate-work.* It is difficult to be sure of this plate-work when the nebenkern is viewed "in the round," but I have tried to represent a small tangential slice of it in Fig. 4. The plate is more or less wrinkled and apparently perforated in places, which irregularities seem to correspond with the points of indentation, folding, or union, of the various plates, as can be explained by a comparative study of Figs. 4, 5 and 6. In the Hemiptera, therefore, a spireme seems not to occur at this stage, and the patterns are due not to a tangled thread-work but to a more or less regular plate-work.

It is necessary now to retrace our steps to the early stages in the formation of the nebenkern, and attempt an explanation of these results from a broadly comparative standpoint. It will be recalled that the peculiar pattern in the lepidopteran nebenkern owes its origin to the arrangement of the chromophilic and the chromophobic constituents of the original granular mitochondria. The question then arises: are the patterns just described in the Hemiptera comparable in any way to those in the Lepidoptera? I believe that all the facts point to an affirmative answer to this question.

It will be recalled that from a comparison of various morphological types of mitochondria the assumption seemed to be warranted that they are all alike composed of two fundamental and distinct substances—a chromophilic material forming the outer covering of each chondriosome unit, and an internal chromophobic material. Assuming this structure to be present in the thread-like type of mitochondria, the transformations of the hemipteran spermatid are readily explicable. During the early stages in the formation of the nebenkern, the presence of the two mitochondrial materials can not be discerned any more than was possible in the auxocyte period. It is, therefore, impossible to say exactly how the fusion of the threads takes place, as Gatenby has done in the case of the vesicular mitochondria. It seems probable, however, that, as in the latter case, there is a process of running together going on within the chondriosome mass, which finally results in the production of sufficiently large groupings of the two materials to allow of a visible separation. This condition is attained in the stage of Fig. 1, in which the chromophobic material has run together to form many small, clear masses or vacuoles, the walls of which are formed, as it were, of the chromophilic substance. The process of condensation which has produced this initial differentiation now continues rapidly (Fig. 2), larger and larger aggregates of chromophobic material being produced by the further condensation of the chromophilic substance. This condensation has seemed, from the beginning, to progress from without inward, so that the primary vacuolization is in the form of concentric shells of vacuoles, each equal in thickness to the diameter of a single vacuole. Thus by the absorption of the partitions of chromophilic material which at first separate the vacuoles of any one layer, there is produced a very typical figure. The chromophilic material has accumulated in a thin lamella between each adjacent layer of vacuoles, while the vacuoles themselves have run together to form a more or less homogeneous ground substance which surrounds, and fills in all the interspaces between the chromophilic lamellæ. These lamellæ seem to be more or less inter-connected and wrinkled, and are possibly perforated in numerous places, producing a sort of skeletal platework, the main outlines of which are molded on the original vacuolization of the nebenkern. The "spireme" of Gatenby ('17) and the "plate-work" which I have described are thus directly comparable in every way. Their apparent difference depends, it would seem, on the original form and method of fusion of the spermatid chondriosomes, which are in one case large, vesicular granules and in the other long, delicate threads.

I believe, therefore, that, depending on the primary morphology of the auxocyte chondriosomes and possibly other unknown factors, the nebenkern may develop a "spireme" structure or a system of plates (or possibly other structural types not yet described). Gatenby ('18) on the other hand seems inclined to the view that all the nebenkern structures are essentially a threadwork comparable to the "spireme" in Lepidoptera. He states, in fact, that a "very finely-coiled spireme" does occur in *Tenebrio,* and presumably other Coleoptera. It is always possible, as Gatenby has shown in the Lepidoptera, that faulty technique will make one of these types look like the other. This may possibly have occurred with some of the Orthoptera, for I find in my own material of *Rhomaleum*, nebenkern figures comparable to those figured by Meves ('00) in *Pygæra*, and yet Giglio-Tos and Granata ('08) figure in *Pamphagus* a very clear "spireme." Of even more interest are the old figures of the nebenkern in *Gryllus* by Baumgartner ('02), in which a "striated condition" very reminiscent of Gatenby's "spireme" is a conspicuous figure. It is, therefore, by no means improbable that the "spireme" type of nebenkern is more widely distributed than has been suspected. On the other hand I am equally convinced that the "plate-work" type of nebenkern is a reality, and it is scarcely probable that technique of so many kinds (I have tried a great many methods on these Hemiptera (see Bowen ('22))) would fail to reveal a "spireme" in the hemipteran nebenkern if any such structure really existed. The whole series of stages which I have described are hardly to be satisfactorily explained away as artifacts. It is, I think, safe, then, to conclude that there are at least two fundamental types of nebenkern structure—the "spireme" type, and the "plate-work" type—possibly interconnected by intermediate conditions; but until the matter has been carefully examined it will not be possible to classify the various insect groups (other than Lepidoptera and Hemiptera) on the basis of nebenkern type. This whole subject offers an attractive field for intensive study.

It remains now to follow out the later history of the chromophilic substance. In the spireme type the chromophilic thread often withdraws from its originally peripheral position, and, after imperfect fixation, the running together of its substance gives rise to the appearances figured by Platner ('89) and Meves ('00) and already referred to above. The final fate of the spireme is not known in any case. In the Lepidoptera the nebenkern does not divide (according to the current descriptions) as it does in the Hemiptera and many other insects, but merely draws out along the tail filament for which it acts in the rôle of a mitochondrial sheath. According to Gatenby ('17), as this elongation is going on, "the chromophobe substance 'dwindles,' and the spireme appears to break up partially." In this unsatisfactory manner the account closes. The probable fate of the spireme will be touched on in a later paragraph.

To return now to the plate-work in the nebenkern of Hemiptera. The process of condensation having reached the stage of Figs. 5 and 6, the plate-work begins now to become much more open and irregular; and in the immediately succeeding stage the resemblance to an onion has been largely lost (Figs. 7 and 8). The running together of the chromophilic material has resulted in the disappearance of a part of the plate-work, which now consists of a few much-folded plates whose optical cross-sections are usually rather simple. Fig. 7 is an especially striking one, since in this case the outline forms a perfect spireme reminding one of Gatenby's account. Fig. 8 is another example of the same stage, and by changing the fine adjustment slightly, the optical section can be converted into a perfect spireme. By a similar change of focus, the spireme of Fig. 7 can be transformed into an appearance similar to that of Fig. 8. If further proof of the plate-like rather than thread-like structure is necessary, it can be provided by a cross-section of the nebenkern (Fig. 9). (Figs. 8 and 9 are from the same cyst of spermatids.) Comparing this cross-section with Fig. 6, it is clear that the chromophilic substance is still arranged in a plate-work form, but now a very irregular one. It is also of interest to note that the plate-work seems to end at opposite points on the surface of the nebenkern, thus marking the future division plane of the mass as a whole.

At a slightly later stage (Fig. 11) the plate-work has become still more condensed and simplified, while the chromophilic substance of which it is composed seems to be decreasing in actual quantity. At this time, as is shown also by cross-sections (Fig. 12), the plate-work forms a flattened, ovoidal core, the exterior of which is now delimited by a smooth, regular layer. Internally a single, much-folded septum is inserted in this outer layer along the future division plane of the nebenkern. The further condensation of the plate-work now progresses rapidly, the ovoidal core of chromophilic substance shrinking considerably in size while the folded, internal septum smoothes out to form a flat, regular partition (Fig. 13). If the spermatid be viewed at right angles to the plane of division of the nebenkern (*i.e.,* at right angles to the plane of Fig. 13), the central chromophilic mass appears as a single, regular plate (Fig. 14).

Slightly later the chromophilic core has shrunk still further (Fig. 15) and finally it becomes so much reduced as to be barely visible as a purple-staining (after Benda) patch within the nebenkern proper (Fig. 16). As the nebenkern begins actively to elongate, the last remnant of the chromophilic material vanishes (Fig. 22) and no trace of it can be found at any subsequent stage. It has disappeared apparently by a process of gradual solution in the chromophobic substance.

The gradual breaking down and disappearance of the "onion" stage has been noted by several previous workers; and still others have figured several of the later stages without apparently any very accurate idea of what was occurring. Henking ('91) described the process in *Pyrrhocoris,* the layers of the onion stage gradually disappearing from without inward by fusing together, leaving at length a small, dark point in the center of the nebenkern which likewise disappears. Gross ('07), working on the same form, has given a slightly different account, less accurate in some respects than that of Henking. Boring ('07) has clearly figured (Figs. 304 and 313) the intermediate condition of my Figs. 7 and 8 in *Amphiscepa* and *Pœciloptera,* while the very regular arrangement of the chromophilic substance in its later phases of dissolution is well figured by Doncaster and Cannon ('20) in *Pediculus.* It is of interest in connection with these last named observers, that they made a conscious effort to find a "spireme" like that described by Gatenby, but failed entirely. Their figures indicate a course of events similar to that in the *Pentatomidæ.* Many other workers have figured what appear to be these later stages in the history of the chromophilic substance, but these need not be dwelt upon here since it is uncertain whether they are to be considered as the end stage of the plate-work type of nebenkern or an artifact from a spireme structure. These figures have the common characteristic of a clear peripheral envelope containing a chromophilic central mass. (See, for example, the figures of Holmgren ('02) (*Silpha*), Vejdovský ('12) (*Diestrammena*), Shaffer ('17) (*Passalus*), and Zweiger ('07) (*Forficula*).)

One further feature which seems to have some relation to the final disappearance of the chromophilic core remains to be considered, viz., the division of the nebenkern into two parts. It will be observed (Fig. 13) that the nebenkern commences to elongate before the chromophilic substance has entirely disappeared, and at the same time there is a marked indication of the longitudinal splitting of the nebenkern into two parts. This division plane seems to deepen as the chromophilic core withdraws more and more toward the center of the nebenkern (Fig. 15), but the division never extends through the core itself. This is clearly shown in Figs. 21*A* and *B*, which show optical cross-sections of the nebenkern at about the stage of Fig. 15, at two different levels. In Fig. 21*A*, the section passes through the chromophilic substance, while in Fig. 21*B* it passes above (or below) it. It is clear that the actual division of the nebenkern has extended only to the periphery of the central, chromophilic mass. When this finally disappears, the division of the nebenkern is completed (Fig. 22), and from this point on the nebenkern draws out as two separate bodies—the mitochondrial sheaths of the tail filament—as described by many authors. It is interesting to note that Henking ('91) observed, after the final condensation of the "pattern," that the two halves of the nebenkern became more and more *sharply set apart* from each other. Thus it appears that the division of the nebenkern waits upon the final disappearance of the chromophilic material, the completion of which process signalizes the final splitting of the nebenkern into two parts.

If there is any real connection between these two phenomena, we should always expect the disappearance of the chromophilic material before the division of the nebenkern. Accordingly, if the absorption of the chromophilic substance is for any reason delayed beyond the initial phases in the drawing out of the nebenkern as a whole. we should expect the nebenkern to elongate as a single mass dividing eventually into two parts at a stage in the elongation dependent upon the time of the disappearance of the chromophilic core. The first of these expectations receives a remarkable fulfillment in many insect forms, and I am not aware that the sequence is ever certainly violated. The Lepidoptera, as described by Meves ('00) and Gatenby ('17) are particularly good examples, and many Coleoptera exhibit a similar condition. Thus, in *Silpha,* Holmgren ('02) figures very clearly the gradual disappearance of the chromophilic material as the undivided nebenkern draws out, and in cross-sections very similar to those which I have figured, he shows that the division furrow of the nebenkern extends only to the periphery of the chromophilic core (his "Mitochondrienrestkoerper"). Shaffer ('17) figures a similar stage in *Passalus,* which he seems to think is divided. From the position of the nebenkern parts, I think, however, that the conditions are like those in *Silpha,* Shaffer having confused the indications of division with the accomplished fact. Still other cases might be cited, all of which tend to support my contention that the division of the nebenkern is never completed until the chromophilic substance has disappeared. It remains, then, to inquire whether, in these cases of delayed division, the disappearance of the chromophilic material is followed by the definite splitting of the nebenkern. The evidence on this point is very scanty since, if division is long delayed, it would usually be overlooked. However, in the case of *Silpha* (Holmgren ('02)) the whole history of events is clear, and Holmgren's figures show conclusively that the nebenkern completes its division after the disappearance of the chromophilic substance. In the case of *Passalus,* if my interpretation of Shaffer's Fig. 21 be correct, the nebenkern is subsequently divided, as shown by his Fig. 20*c,* though the exact relation to the disappearance of the chromophilic material is not established. The classical case of the lepidopteran nebenkern has never been followed out in detail, and it would seem that the chromophilic material retains its identity for a long period. But I will venture the guess that in this case, also, a division of the nebenkern will be found to occur once the chromophilic substance has been disposed of.

This completes the history of the origin and nature of the "blackberry" and "onion" patterns which were mentioned in the beginning of this paper as two characteristic types in the insect nebenkern. It remains now to consider the nature of the third type of pattern—the smooth or beaded cords running parallel to the long axis of the nebenkern as mentioned in the paragraph referred to above. This type of pattern has been described especially by Holmgren ('02) and Vejdovský ('12). Fragmentary references to it occur, however, in a number of other cases, as for example those of *Pygæra* (Meves ('00)) and of *Notonecta* (Pantel and de Sinéty ('06)); Henneguy ('96) also seems to have figured it crudely in *Pyrrhocoris,* and Boring ('07) shows it very clearly in *Pœciloptera*. In so far as any explanation of this pattern has been attempted, all accounts agree in connecting it ultimately with the preceding patterns, that is, with the chromophilic substance. This is certainly not the correct origin in the *Pentatomidæ;* in fact, we have here to deal with a structure of entirely independent (so far as its morphology is concerned) origin. Indeed, it is better to consider this from the standpoint of the evolution of a new substance within the nebenkern, rather than as a mere pattern comparable to those which have preceded it.

This substance, then, makes its first appearance at a period approximately that of Figs. 7, 8 and 9—in other words at about the time when the chromophilic substance first seems not only to be condensing but actually in process of dissolution. This new material appears at this time in the form of vacuoles scattered about in the chromophobic substance between the chromophilic plate-work and the peripheral wall of the nebenkern (Figs. 7, 8 and 9). These vacuoles are rather inconspicuous at first, their periphery staining faintly in Benda preparations, though I am not able to say positively whether the alizarin or the crystal violet is responsible for the coloration. It seems to be the former as a rule. The origin of these vacuoles is entirely obscure. Perhaps they are produced as a special differentiation of the chromophobic material. However, the fact that these vacuoles are on the increase while the chromophilic material is decreasing suggests that there is possibly some connection between the two processes. It might be supposed that the vacuoles are differentiated out of material derived from the chromophilic mass in some indirect way.

Whatever the origin of these vacuoles may be, they increase rapidly in number, and soon become arranged in thread-like rows running parallel to the future long axis of the nebenkern, subsequently becoming more or less fused together to form structures reminding one of strings of beads (Fig. 11). In cross-section, these threads appear as single vacuoles, the position of which marks the distribution of the threads (Fig. 12). In immediately subsequent stages (Figs. 13 and 15) these beaded threads become still more conspicuously developed, and as the chromophilic substance disappears they become distributed throughout the chromophobic envelope as shown by cross-sections of the nebenkern (Figs. 21*A* and *B* and 22).

The account, thus far, is taken from Benda preparations, in which the vacuoles stain faintly as described above. Occasionally, however, the substance itself of the vacuoles stains more or less darkly with the crystal violet (right half of Fig. 11), becoming much more conspicuous than is usually the case. Very frequently these vacuoles are not demonstrated at all by the Benda method, the chromophobic material appearing entirely clear. The above account applies particularly to the *large-cell generations;* in the *small-cell generations* (in *Murgantia* the large-cell generations also are not sufficiently large to make any difference in this respect) the results with Benda are quite different. In these the crystal violet often acts rather capriciously, but as described by many authors it usually *stains the whole mass* of the nebenkern an intense violet. In other words, the *chromophobic* material takes the *violet stain.* This difference in staining behavior of the large and small nebenkerns, so fortunate for this investigation, is probably due to some physical factor which results in the ready extraction of the stain from the large nebenkern masses while from the small ones the stain is usually extracted with more difficulty. In the latter case, as in the former, the substance of the newly formed vacuoles fails to take the violet stain in good preparations, and thus the substance of the nebenkern appears to be composed of two materials of very different staining capacities. I thought, at first, that the material which thus takes the crystal violet (in the small-cell generations) was equivalent to the outer, chromophilic substance of the original mitochondria;[1] but the history which I have traced shows clearly that exactly the reverse is the case. So much for the dangers of arguing on the basis of a single staining reaction. As a result of this behavior toward crystal violet, the nebenkern appears vacuolated in various characteristic ways, the vacuoles being at first scattered, and subsequently arranged in longitudinal rows that seem to be fusing to form a non-staining axis for the outer, intensely staining substance. (See Bowen ('22) Figs. 59, 60 and 66.)

The most interesting results, however, were obtained with Cajal's formol-uranium nitrate method with which I had been attempting the demonstration of the Golgi elements. This technique gave no certain results in the very early stages of the formation of the new, or as we may conveniently refer to it, the *central,* substance, but when the vacuoles had lined up to form thread-like chains (about the stage of Fig. 11), the silver was very heavily reduced by the substance of the vacuoles themselves which were thus brought out with the clearness of a silhouette on a field of bright canary yellow (Fig. 10). The general fixation of the spermatid is usually very poor, the nucleus appearing merely as a light, circular area, often badly distorted. But the nebenkern is well preserved and the beaded threads stand out with remarkable clearness, especially in later stages. At this early period they are not quite so clear on account of the mass of chromophilic material which still occupies the center of the nebenkern and is colored a nondescript brown. Whatever may have been the origin of the substance of these threads, it is abundantly clear that it represents now a material of specific and characteristic chemical composition. I had thought at first (Bowen ('19)) that it represented the original chromophobic material of the chondriosomes, but it is now evident that such can not be the case.

As the nebenkern draws out, the threads make for a time a rather confused picture since the sections rarely cut them exactly parallel to their length (Fig. 17 is a fair example). The cross-sections of these stages are more interesting, the threads, now cut across, appearing with diagrammatic clearness (Fig. 20). In early stages I have counted at least 15 to 20 such thread sections in a single half of the nebenkern. At this same stage Kopsch preparations are sometimes obtained in which the chromophobic substance is very intensely blackened while the threads are left clear. Thus a picture exactly the reverse of the Cajal result is obtained. (Compare Figs. 17 and 20 with Figs. 18 and 19.) With the elongation of the nebenkern the threads spin out along its course and show a progressive decrease in number, as can be clearly demonstrated in cross-sections (compare Figs. 20 and 24), as well as in plane views (Fig. 23) of the nebenkern. They now show the beaded appearance perhaps due to their origin from chains of separate vacuoles, in a very characteristic manner. It is not easy to say at this stage just how the reduction in the number of threads takes place, but there appears to be a side by side fusion of adjacent threads, a single thread thus arising from two preceding ones. This fusion goes on rapidly until at the stage when the tail vesicles (see beyond) are first formed, the threads of each half of the nebenkern have fused to form a single axial core for their respective nebenkern portions (Figs. 25 and 26). In the final condensation of the threads proof of the lateral fusion of the separate threads can be obtained; for in stages just prior to that of Fig. 26 the threads will, in some places along their course, be already fused to form a single axial core, while in other places they are still clearly separate. Paulmier ('99) noted this central core in the elongating nebenkern halves of *Anasa,* likening it to "a long vacuole extending down each one."

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[1] It should be remembered that the true chromophilic substance disappears entirely at a relatively early stage, as described above.

The origin of these threads (central substance) seems to have been studied only by Holmgren, and Vejdovský. In *Silpha,* according to Holmgren ('02) there is, in each half of the nebenkern, a single, beaded thread, which arises from the central chromophilic mass by a process of splitting off. To these thread-like portions thus separated off is given the name of "Mitochondrienbalken." Slightly later the chromophilic mass disappears as I have already noted above, and the threads now occupy the halves of the divided nebenkern exactly as I have described the arrangement in Hemiptera. Holmgren figures also a number of cross-sections which are very similar to mine, except that there is never more than one thread in each half of the nebenkern at any stage. Subsequently the chromophobic material is said to disappear and the threads themselves finally dissolve leaving no trace of mitochondrial substance in the mature sperm. This account of the fate of the threads and the nebenkern substance as a whole is negatived by more recent studies on the rôle of the mitochondria in sperm formation. As to the mode of origin described by Holmgren, it seems to me not improbable that technical faults caused him to overlook the early history of the threads, and at the time when he first demonstrated them their development was already well advanced.

In *Diestrammena,* according to Vejdovský ('12), the nebenkern has at first the characteristic division into a central chromophilic substance and an enveloping zone of chromophobic material. Cross-sections of this stage are quite similar to mine except that the axial filament is represented as piercing the center of the chromophilic core. This is a very unusual condition, if true, the customary position of the filament being in or near the cleft of the nebenkern, but outside of the substance of the nebenkern itself. (Compare my Figs. 21*A* and *B,* and 22 with Vejdovský's Figs. 181*e, f, g.*) Presently there appears in the chromophilic material a number of separate, intensely staining granules which become arranged in rows parallel to the axial filament; and eventually the chromophilic material is entirely replaced by spirally wound (later beaded) threads. The end result is strikingly like that in the Hemiptera. Vejdovský believes that the process is to be interpreted as a transformation of the "Chondriom" (chromophilic substance) into the granular components ("Mitochondrien") from which it originally arose. According to this view the threads would be secondarily a differentiation product of the chromophilic material itself—a view somewhat similar to that of Holmgren. The beaded appearance of the threads marks, according to Vejdovský, the breaking up of the threads, the granular products (Mitochondrien) thus formed swelling up to a large size and being simultaneously transformed into a fat-like substance. These fat droplets seem to fuse together to form larger aggregates, at the same time *absorbing the chromophobic material,* with the final result that the axial filament lies naked in the cytoplasm with the fat droplets scattered along its course. These later pass out along the tail to be collected in the protoplasmic masses that are sloughed off in the final maturing of the sperms, which, as a result, possess no trace of mitochondria or their derivatives. This again reminds one of Holmgren's account, and the same comment relative to the findings of other authors is applicable by way of criticism.

In view of the unusual fate ascribed by Holmgren and Vejdovský to these final structural components of the nebenkern, it is of interest, finally, to inquire into their later history in the Hemiptera. In stages subsequent to that of Fig. 26. the Cajal method fails to impregnate the central substance of the mitochondrial sheaths, which continue to draw out rapidly with a consequent decrease in their diameter. The only evidence which I have been able to get on the structure of the nebenkern in these later stages has been obtained from Benda preparations. I have already called attention to the fact that in *some* cases (*e.g., Murgantia*) I have obtained preparations in which the outer, chromophobic material stained intensely with crystal violet, while the substance of the vacuoles (the central substance) remained colorless. Accordingly, when the substance of the latter has run together to form a single axial core for each half of the nebenkern (Fig. 26), the two substances can still be distinguished, the outer layer (the chromophobic portion) staining a deep purple while the central core (the central substance) is unstained. (See Bowen ('22) Figs. 76 and 78.)[1] As I have described in another paper (Bowen, ('22)) the mitochondrial sheaths begin now to develop characteristic vesicles, the intervening substance being simultaneously spun out into thin connecting threads. The result (Fig 27) is that each of the sheaths becomes spun out into a long, delicate thread along the course of which these vesicles are scattered as bleb-like swellings. The structure of these swellings is very clearly demonstrated by cross-sections of the tail. In such sections (Fig. 28) the sheaths themselves appear as small dots intensely stained with crystal violet, while the vesicles appear as masses of non-staining material enclosed by a peripheral layer which stains like the portions of the sheaths which intervene between two adjacent vesicles. It is as if the thread-like sheaths were inflated at intervals to form little pockets in which is placed a small mass of some material staining quite differently from the substance proper of the threads. My impression is that these vesicles are eventually smoothed out, their substance being perhaps distributed evenly along the thread-like mitochondrial sheaths. Needless to add, these sheaths are not broken down as stated by Holmgren and Vejdovský. (See Bowen ('22)). It is also clear that it is these vesicles which Vejdovský has confused as the "fat droplets" derived from the breaking down of the mitochondria as a whole. Obviously, their real nature is entirely different.

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[1] It may be as well at this point again to call the attention of the reader to the confusing differences in the staining behavior of the nebenkern substances. In the first place, it is to be noted that the *central substance* almost invariably fails to take the crystal violet of Benda's method, while the *chromophilic material* is always stained violet if the nebenkern retains the stain at all. The *chromophobic material,* however, behaves in a variable way. Under the best conditions it does not retain the crystal violet (at least in early spermatid stages) when the rest of the preparation is properly differentiated; but sometimes in the early spermatid stages (small-cell generations), and invariably in the later ones, it stains heavily with the violet—*an exact reversal of staining behavior.*

The exact origin of these vesicles has not been satisfactorily made out, and the relation of their structure to that of the earlier stages is therefore uncertain. It is possible, however, that the central substance of the nebenkern derivatives tends to collect at intervals along the course of the sheaths, thus forming the bleb-like swellings, while in the intervening lengths, from which the central substance is withdrawn, the sheath is correspondingly thinned out (Fig. 27). Possibly such accumulations of material play some rôle in the spinning out of the sheaths. The ultimate fate of this central substance is unknown; possibly it comes finally to form a delicate core for each of the thread-like mitochondrial sheaths. Thus is completed the long and complicated differentiation of the spermatid nebenkern.

Conclusion.

One of the most interesting results of this study has been to reveal the nebenkern as the seat of an unexpectedly complicated and determinate series of differentiations. Instead of an inert, passive mass of mitochondria subject only to some enigmatic structural "patterns," we find that the nebenkern is actually the seat of intense activity up to a very late stage in sperm formation. What the meaning of this may be, is, however, entirely unknown at present.

A second point of very practical nature is the light thrown upon the staining behavior of the mitochondrial substance during spermiogenesis. It is clear that in preparations which have every appearance of technical excellence, the structure of the nebenkern may be seriously distorted or entirely invisible. This has obviously been the cause of many incomplete accounts, and of much contradiction of results even in the same animals. It now becomes plain that the exact study of the nebenkern demands the use of a great variety of technical methods in the hope that one of them will prove adequate for the work in hand. Similarly, the obvious changes in the chemical composition of the nebenkern substance arouse a well-grounded skepticism as to the value of the so-called "specific" stains for mitochondria. In a mass of changing chemical composition such as I have shown the nebenkern to be, it is manifestly impossible to talk of any "specific" staining at all; and equally impossible to assign a chemical composition to this chondriosome body as one might to a homogeneous mass.

I have tried in this paper to put into a logical and connected story the facts as we know them about the spermatid nebenkern in insects. In no single form have all the features of this account been, as yet, clearly made out, and my interpretation of the facts as I have found them in the Hemiptera involves, therefore, a large element of assumption. I have, however, felt justified in these assumptions since they furnish a satisfactory basis of explanation for all the facts so far available. many of which have been entirely obscure hitherto; and since, furthermore, they provide a standpoint from which a further, intelligent attack on the whole problem can be made.

By way of summary, attention may be particularly called to the following points:

1. The nebenkern of the insect spermatid is composed primarily of two distinct substances—a chromophobic ground-substance presumably equivalent to the medullary material (of like staining behavior) in the original chondriosomes; and a chromophilic substance presumably equivalent to the peripheral material in the original chondriosomes.

2. The chromophilic substance is arranged in a variety of ways producing the so-called nebenkern "patterns."

3. These patterns are presumably of at least two types—(1) the "spireme" type characteristic of Lepidoptera, and (2) the "plate-work" type characteristic of Hemiptera.

4. The chromophilic substance eventually disappears apparently by some process of condensation and solution.

5. As the chromophilic substance disappears, a new substance, possibly a differentiation product of the chromophilic material, makes its appearance within the mass of chromophobic stuff.

6. This new (central) substance forms a core for each of the elongating halves of the nebenkern, and possibly is the source of the bleb-like swellings later developed on the tail sheaths.

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EXPLANATION OF PLATES.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3,800 diameters. At so great an enlargement it has, of course, been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 3,000 diameters. In every case the method employed in the preparation of the original object has been indicated.

Plate I.

Fig. 10 is from *Euschistus euschistoides;* the others are from *Brochymena quadripustulata.* All the figures are from cells of the large generations.

Fig. 1. Early spermatid with first indications of nebenkern differentiation. (Benda.)

Fig. 2. Slightly later stage than the preceding, showing development of the peripheral, chromophobic zone. (Benda.)

Fig. 3. Peripheral, chromophobic zone completed and early differentiation of the chromophilic plate-work. (Benda.)

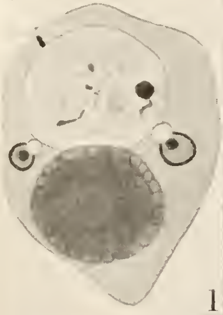
Figs. 4, 5, 6. Intermediate stage in the development of the chromophilic plate-work. Fig. 4 is a surface view of a small slice of the nebenkern plate-work. Fig. 6 is a cross-section of the nebenkern at right angles to the major axis of the spermatid, the axial filament appearing below in cross-section. (Benda.)

Figs. 7, 8, 9. Early stage in the final condensation of the chromophilic plate-work and the first appearance of the vacuoles (central substance) in the chromophobic material. Fig. 9 is a cross-section of the nebenkern made like Fig. 6, the tail filament appearing below. (Benda.)

Fig. 10. Early stage in the running together of the vacuoles in the chromophobic material to form beaded threads. (Cajal.)

Figs. 11, 12. Slightly later stage than the preceding, showing intermediate stage in the final condensation of the chromophilic material. Fig. 12 is a cross-section of the nebenkern made like Figs. 6 and 9. The beaded threads are seen in the chromophobic material. (Benda.)

Figs. 13, 14. Later stages in the disappearance of the chromophilic plate-work. Fig. 14 is at right angles to the plane of Fig. 13. (Benda.)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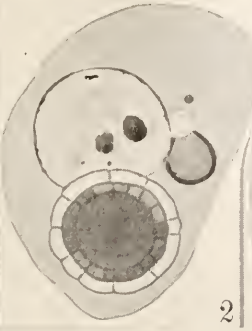
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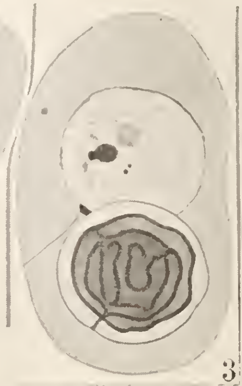
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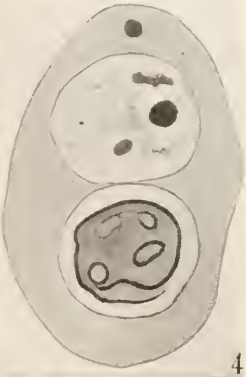
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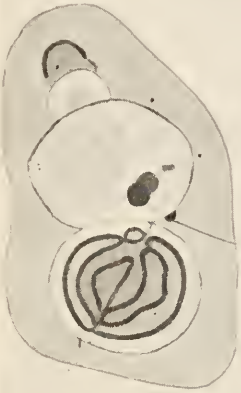
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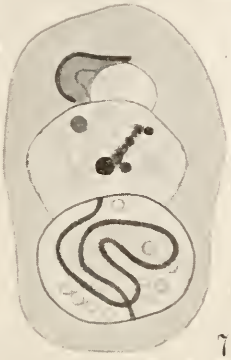
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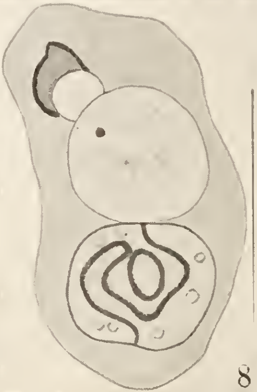
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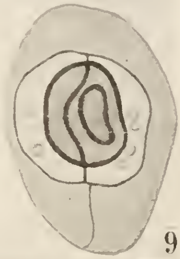
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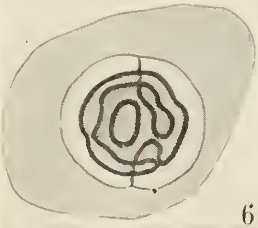
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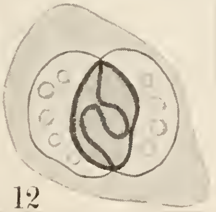
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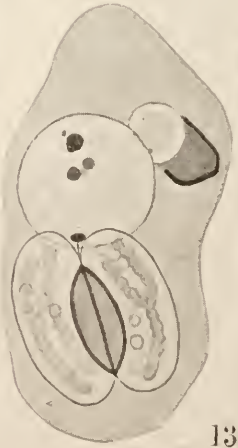
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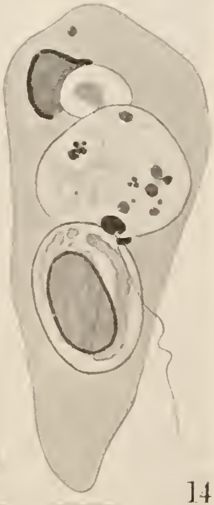
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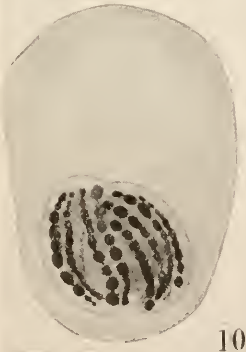
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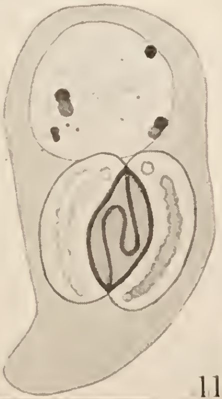
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R. H. BOWEN.

Plate II.

Figs. 27 and 28 are from *Murgantia histrionica;* Figs. 15, 16, 21*A*, 21*B* and 22 are from *Brochymena quadripustulata;* the others are from *Euschistus euschistoides.* All the figures are from cells of the large generations.

Fig. 15. Late stage in the disappearance of the chromophilic substance. (Benda.)

Fig. 16. Final stage in the disappearance of the chromophilic substance. (Benda.)

Fig. 17. Early stage in the drawing out of the nebenkern, showing the vacuoles and beaded threads (central substance) which have recently appeared in the chromophobic substance. (Cajal.)

Figs. 18, 19. Same as Fig. 17. Fig. 19 is a cross-section of the nebenkern at right angles to the major axis of the spermatid. (Kopsch.)

Fig. 20. Cross-section of the nebenkern made like Fig. 19, at a stage slightly later than that of Fig. 17. (Cajal.)

Fig. 21. Cross-sections of the nebenkern made like Fig. 19, approximately at the stage of Fig. 15. *A,* through the level of the chromophilic plate-work; *B,* above (or below) the level of the chromophilic plate-work. (Benda.)

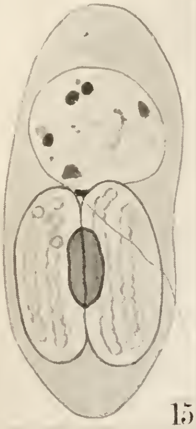
Fig. 22. Cross-section of the nebenkern made like Fig. 19, after the final disappearance of the chromophilic substance. (Benda.)

Figs. 23, 24. Intermediate stage in the lateral fusion of the threads of central substance. Fig. 24 is a cross-section of the nebenkern made like Fig. 19. (Cajal.)

Fig. 25. Portion of the mitochondrial sheaths at about the stage of Fig. 26. At one end the sheaths were turned upward and so cut in cross-section. (Kopsch.)

Fig. 26. Final stage in the condensation of the central substance; the threads of Fig. 23 have fused to form a single, axial core for each of the nebenkern halves. (Cajal.)

Figs. 27, 28. Development of the bleb-like swellings on the mitochondrial sheaths. Fig. 27 is a small portion of the tail region near the head of the sperm. Fig. 28 is a cross-section of three sperm tails at the stage of Fig. 27. (Benda.)



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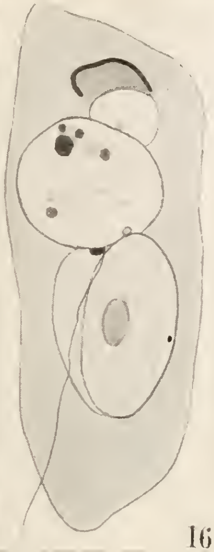

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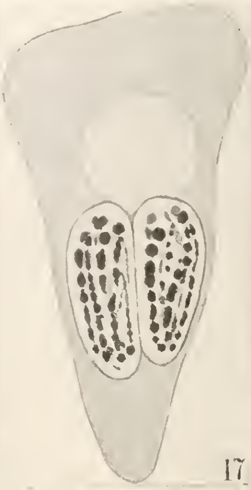

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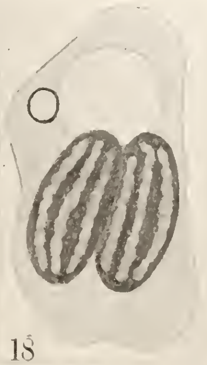

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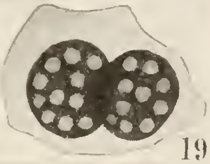

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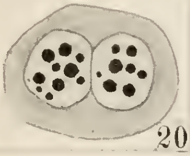

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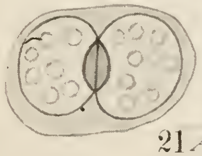

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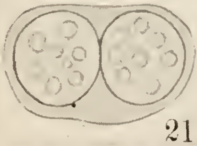

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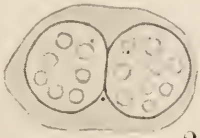

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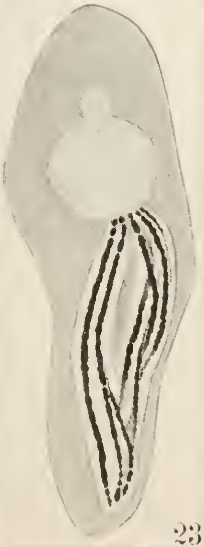

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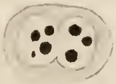

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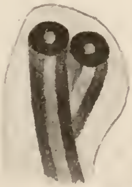

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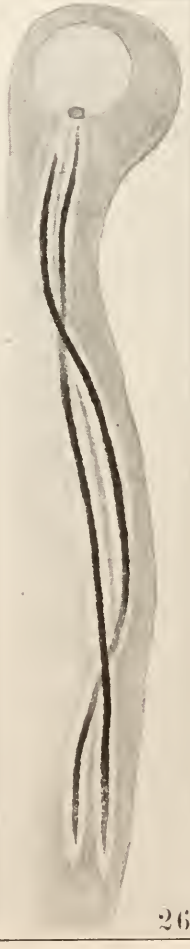

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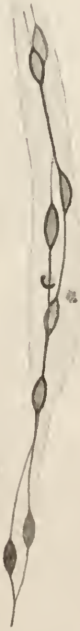

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R. H. BOWEN.

A DETERMINATION OF THE DEGREE OF CONSTANCY IN THE NUCLEI OF CERTAIN ORGANS IN HYDATINA SENTA.[1]

H. J. VAN CLEAVE.

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INTRODUCTION.

As a result of his extensive studies on the cytology of *Hydatina senta,* Eric Martini (1912: 631) maintained that each individual of this rotifer bears a constant total number of 959 nuclei distributed in fixed numbers through the various organs of the body. In other publications, Martini has mentioned the fact that data concerning variability encountered in his studies of cell-constancy have been too meagre to permit of quantitative study. His published results on cell-constancy in *Hydatina* contain no reference to the numbers of individuals examined in reaching his conclusions of definiteness in cell or nuclear numbers. A. F. Shull (1918), in an attempt to determine the validity of Martini's claim of constancy in histological elements, has made a statistical study of the nuclei in the vitellaria and gastric glands of *Hydatina.* Contrary to the findings of Martini, Shull has recorded a relative "inconstancy" in the numbers of the nuclei in the organs which he investigated. Thus, Shull failed to find eight nuclei an immutable condition for the vitellarium, for in 4 per cent. of his 245 specimens observed an aberrant number was recorded.

Immediately following the appearance of Martini's monograph the present writer became interested in checking over certain minor details of his findings from materials which had been prepared for class study. A preliminary examination of more than 100 individuals of *Hydatina* failed to reveal any deviation from the usual numbers of nuclei in either the vitellarium or the gastric glands. The same slides were used in a laboratory class in advanced invertebrate zoölogy composed chiefly of university juniors and seniors. For six years each member of this class, averaging about 15 students per year, was required to note and report upon the numbers of nuclei found in the vitellaria of specimens examined. The assignment has always been worded in such a manner as to place a premium upon the discovery of unusual numbers of nuclei, without giving the students any previous hint of the work on cell-constancy. There has never been a verified instance of other than eight nuclei reported by members of these classes. Rather frequently nine nuclei have been reported but in every instance in which a check has been made the ninth nucleus has been demonstrated to belong to the adjacent ovary and readily distinguishable from the nuclei of the vitellarium. The data included in these student observations have not been included in the tabulated results presented later in this paper but comprise a very considerable bulk of evidence of the infrequency of variations in the numbers of nuclei in the material under consideration.

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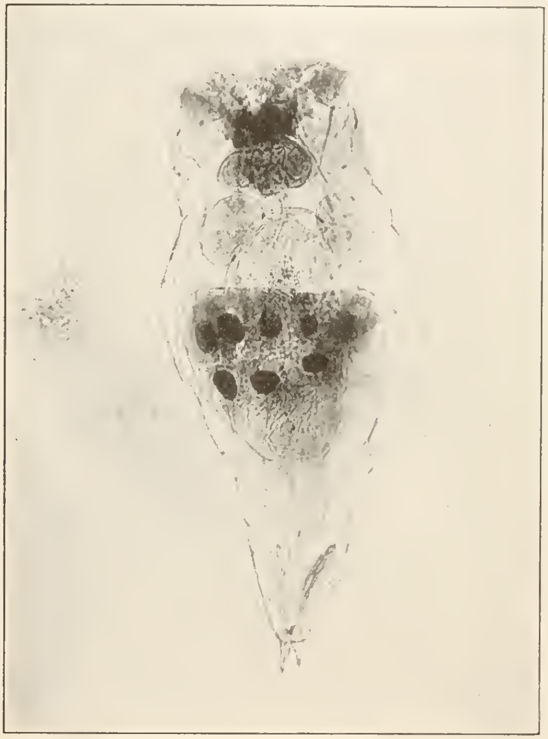
[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 192.

Stimulated by the publication of conflicting observations by Shull, the writer has decided to bring together and to extend his original observations on this phase of the problem of the nuclear numbers in *Hydatina*.

Much of the data available for the statistical study of range in variability are due to the careful assistance of Miss Roselle Karrer who examined 550 individuals of *Hydatina* and by means of a mechanical stage recorded the location of all specimens which displayed peculiarities of any sort. A critical examination of all unusual specimens was made independently by Miss Karrer and the writer, and in no instance was there found any ground for disagreement in the interpretation of the evidence presented by the specimen.

MATERIALS.

The materials upon which these observations are based comprise a series of slides, each containing numerous stained, whole mounts of *Hydatina senta* prepared by Powers and Powers of Lincoln, Nebraska. The excellence of the cytological details displayed by these mounts speaks well for the methods employed in their preparation. Nuclei of the vitellarium, stomach, gastric glands, intestine, and some other structures are readily observable and sharply differentiated from other structures.



TEXT-FIGURE *A*. Photograph of a whole mount of *Hydatina*. The vitellarium is the large gland, with conspicuous nuclei, near the center of the body. The gastric glands show as bean-shaped organs anterior to the vitellarium and attached to the sides of the stomach.

The ultimate source of the cultures from which these mounts were made has not been determined. According to Shull's observations (1918: 460) this does not seem to be of any great importance for deviation in nuclear numbers seems to be inherent in the various cultures examined by him. Age, culture media, and male and female producing strains are the factors specifically considered by Shull in connection with his generalization that there are "no striking differences in the numbers of aberrant glands under different circumstances."

Serial sections were used by Shull in securing his evidence. Numerous sources of probable error are inherent in data resulting from the study of serial sections. Practically all of these are eliminated by the use of well prepared toto mounts. For many features in a work as comprehensive as that of Martini whole mounts would be entirely precluded for the study of finer details of minute structures, but in the study of gross structures such as the vitellaria and gastric glands physical limitations do not preclude the possibility of securing perfect data.

Variability in the Nuclei of the Vitellaria.

In the first 210 individuals examined by the writer each one had 8 nuclei in the vitellarium. At first this was considered as a fair instance of random sampling offering conclusive evidence of absolute constancy in the number of nuclei. Later, in an examination of 560 additional specimens, 3 individuals with aberrant numbers of nuclei in the vitellaria were discovered. Two of these vitellaria possessed 10 nuclei each and the remaining one 12. Thus in 770 specimens examined less than 0.4 per cent. displayed an abnormal number of nuclei in the vitellarium. Contrasting these observations with those of Shull as shown in the following table, two points are especially worthy of note: (1) No individual with less than 8 nuclei in the vitellarium has been observed by the present writer and (2) in no instance has an odd number of nuclei been observed.

Table I.

Frequency of Different Numbers of Nuclei Observed in the Vitellaria of *Hydatina senta*.

Number of nuclei	5	6	7	8	9	10	11	12
Times observed by Shull	1	0	5	235	3	1	0	0
Times observed by Van Cleave	0	0	0	767	0	2	0	1

The plan of the arrangement of the nuclei in the vitellarium of *Hydatina* is not immutable. There is, however, a strong tendency for the nuclei to occur in definite arrangement. Before the gland has reached its full functional stage in development the nuclei (Fig. 2) frequently assume the same definite orderliness of arrangement characteristic of the mature female (Fig. 1). Typically, the 8 nuclei are arranged in the form of a letter V extending across the body with the base of the V at the lateral margin that is contiguous to the ovary. In such instances there is an anterior row of 4 nuclei, a posterior row of 3, and a single nucleus lying near the margin midway between the two rows. This usual arrangement of the nuclei is described because it seems to throw some light upon the question of the interpretation of the aberrant individuals that have been observed in the course of this study.

With but three individuals displaying aberrant numbers of nuclei in the vitellaria the writer realizes that the material is too meagre to permit of broad generalizations. Since, however, all three of these specimens have so many characteristics in common, it seems that the line along which the abnormal condition has been reached must have been fairly uniform. In the two individuals having 10 nuclei in each vitellarium (Figs. 7 and 8), there is strong evidence that the two accessory nuclei have resulted from a division of two nuclei in the region of the ovary. Thus each transverse row contains an additional nucleus. Arrangement of the nuclei in the vitellarium having 12 nuclei (Fig. 9) very strongly supports the hypothesis that this condition has arisen from the typical vitellarium with 8 nuclei through a supplementary division of two nuclei of each transverse row thereby adding 4 nuclei to the normal number. The time at which this division occurred cannot be determined from an examination of the specimens. In any of the three instances mentioned above the non-conformity of nuclear numbers may have been brought about either by the failure of certain nuclei to lose their power of mitotic division at the accustomed stage in the development of the organ or through some sort of stimulation of certain nuclei of a normal mature organ to undergo either mitotic or amitotic division. Evidence observed in the course of this study seems to point toward a late amitotic division occurring in the mature organ.

Since the past history of the specimens under consideration has not been available data concerning the age of individuals and source of the strains are not directly obtainable. Consequently the conditions operating to produce the abnormal glands are not known. In addition to the three instances of abnormal numbers of nuclei in the vitellarium mentioned above, numerous other variations from the usual structure have been observed. Frequently the nuclei are heavily vacuolated (Figs. 3, 4, 5 and 8). An extreme example of vacuolization is shown in Fig. 3 in which one nucleus has attained such a high degree of vacuolization that its chromatic material is highly dispersed and the nucleus greatly swollen.

A more common type of abnormality, possibly associated with advanced age and apparently of significance in considering the problem of increased numbers of nuclei, is that of change in form of the nuclei. Development of irregularity of outline and elongation with accompanying constrictions are two of the more common types of such anomalies. Figs. 4, 5, 6 and 7 show characteristically constricted, lobed, angular, indented, and twisted nuclei. These varying forms all give the appearance of adaptations described in the literature on cytology for increase in nuclear surface necessitated by some change in the normal activity of the gland. In this specific instance no experiments have been performed to determine the conditions responsible for this attempt at readjustment of nuclear surface to meet increased demands. It does seem significant that the changes in form of the nuclei closely resemble conditions observed in other tissues the nuclei of which are undergoing amitotic fragmentation. From nuclei such as shown in Figs. 4 and 6 it is no very great step to a separation of the constricted parts into independent nuclear masses.

Modifications of an identical type have been observed in different genera of Acanthocephala. As shown by the writer (Van Cleave 1914) members of the family Neoechinorhynchidæ display six giant nuclei in the entire subcuticula. In all genera of this family these subcuticular nuclei are definitely limited ovoid or elliptical masses. In members of the genus *Quadrigyrus* (Van Cleave 1920) these same subcuticular nuclei are of two types, those in the anterior region of the body being identical in form with those just described for the Neoechinorhynchidæ while those in the posterior region are coarsely dendritic in nature, composed of an elongated central mass from which a considerable number of projections are given off along the channels of the lacunar system. Nuclei of this second type are distinctly intermediate between the form characteristic of the Neoechinorhynchidæ and those described for the species *Echinorhynchus thecatus*. In this last named species the subcuticular nuclei are finely dendritic, extending as fine branches over a considerable area. In some species of the genus *Echinorhynchus* and of the genus *Pomphorhynchus* this dendritic condition has gone to such an extreme that the small nuclear masses are joined by only fine threads and in many places these communicating threads have not been detected. In this last named condition the nuclear material has the appearance of being distributed in small isolated nuclear masses throughout certain regions of the subcuticula. Thus in this group of parasitic worms the subcuticular nuclei present a finely graded series of changes from the simple rounded or ovoid nuclei retained directly from the embryonic condition to a highly fragmented condition in which there is little or no regularity in the numbers and arrangement of the particles resulting from the fragmentation. Consequently in Neoechinorhynchidæ there is a constancy in the number of the subcuticular nuclei throughout the life of the individual while in the most of the other Acanthocephala late embryonic or post-embryonic fragmentation of the nuclei disrupts the constancy in numbers of histological elements.

That a similar amitotic division might be the explanation of increased numbers of nuclei in the aberrant vitellaria of *Hydatina senta* seems highly probable. Moreover, the abnormal individuals observed in this study seem to indicate that definitely localized stimuli operate in inducing further divisions of the nuclei, for not all of the nuclei of the vitellarium are affected. In all three of the aberrant specimens observed the increase in the number of the nuclei has apparently been in the region of the vitellarium immediately in contact with the ovary. The exact nature of the stimulating agent has not been determined but it seems probable that some change in the vitellarium may be induced by or correlated with changes in the adjacent ovary.

The glands which have 10 nuclei each seem to offer distinct evidence that the additional nuclei have been derived by the division of one nucleus from the anterior and one from the posterior row of a normal individual (Figs. 7 and 8). If two nuclei of each row in a normal individual were to undergo division 12 nuclei would result as is the case in one individual observed (Fig. 9).

Nuclei of the Gastric Glands.

Shull found 9 out of 120 gastric glands which he examined carrying numbers of nuclei other than 6, the characteristic number. According to his records 7.5 per cent. of the glands fail to support the idea of constancy in the numbers of nuclei. By using the same whole mounts described earlier in this paper the writer has secured data on the nuclei of 435 gastric glands, not a single one of which displayed any deviation from the 6 nuclei characteristic for that organ. A comparison of the two sets of data is clearly presented in the following table.

Table II.

Frequency of Different Numbers of Nuclei Observed in the Gastric Glands of *Hydatina senta*.

Number of nuclei	4	5	6	7
Times observed by Shull	1	7	111	1
Times observed by Van Cleave	0	0	435	0

According to Shull's statement (1918: 463) all of the various conditions of culture medium, age, and male or female producing strains seem to produce aberrant numbers of gastric gland nuclei. In the case of male producers he examined only 36 individuals and found none with aberrant numbers of nuclei, but also mentioned the fact that in view of the small numbers examined this circumstance may be insignificant.

Conclusions.

Referring to the solution of the problem of cell-constancy, Shull (1918: 464) has said: "there is no need of complicating this solution by assuming a degree of constancy that does not exist." In speaking of the results of his own work on cell-constancy in *Hydatina* he has maintained that he has obtained "A measure of its exactitude" as expressed in his finding 4 per cent. of individuals bearing inconstant numbers of nuclei in the vitellarium and 7.5 per cent. showing aberrant numbers of nuclei in the gastric glands.

In contrast with these observations stand those included in the present paper wherein a much greater number of individuals were examined, and yielded less than 0.4 per cent. inconstant vitellaria and absolute constancy in the gastric glands.

The complete absence of subnormal numbers of nuclei from the data presented by the present writer seems especially significant in that it indicates that the mechanism operating to bring about constancy of nuclear numbers has operated to its full extent and that any abnormalities represent an inconstancy secondarily imposed. Reductions in nuclear numbers might also result from secondary causes but the writer has seen no evidence of factors operating in the regions studied to bring about a reduction in the numbers of nuclei.

As a result of his observations, Shull seems to have full conviction that the tendency to vary is inherent in the nuclear numbers of *Hydatina* regardless of circumstances such as culture medium and strain. If his conclusions in this regard are valid, either some factor or group of factors not considered by Shull have been operating to suppress the inherent tendency to vary in the particular culture studied by the writer or physical difficulties involved in reconstructing and counting the nuclei from serial sections have introduced an element of error into his results.

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PLATE I.

All drawings were made from whole mounts with the aid of a camera lucida and at a uniform magnification.

The vitellarium is viewed from the ventral surface in all instances except in Figs. 4 and 7, which are drawn from the dorsal surface.

FIG. 1. Vitellarium of normal mature female, showing usual form of the gland and characteristic number and arrangement of the nuclei.

FIG. 2. Very small vitellarium from a presumably young female.

FIG. 3. Vitellarium showing characteristically vacuolated nuclei, one of which has the chromatic substance so much dispersed that it is distinctly paler in color.

FIG. 4. Vitellarium showing lobed, angular and constricted nuclei.

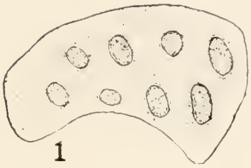
FIG. 5. Vitellarium with peculiarly twisted and cleft nuclei.

FIG. 6. Abnormal vitellarium with greatly elongated and constricted nuclei.

FIG. 7. Abnormal vitellarium with 10 nuclei. In this drawing the ovary is shown overlying the vitellarium on the right side.

FIG. 8. Abnormal vitellarium with 10 nuclei. Part of the ovary is shown underlying the vitellarium on the left of this drawing.

FIG. 9. Abnormal vitellarium with 12 nuclei.



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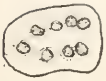

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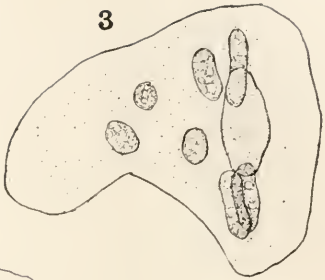

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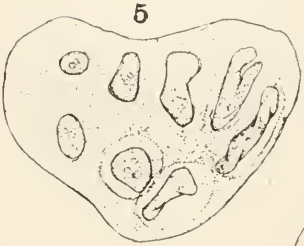

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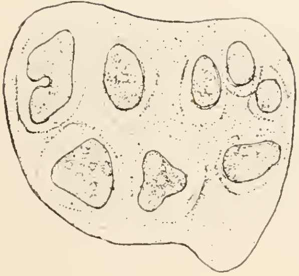

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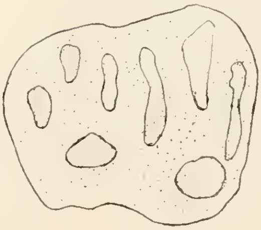

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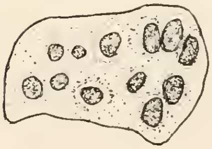

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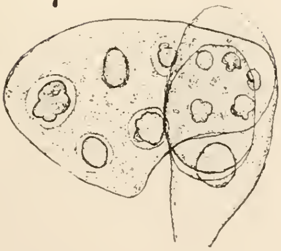

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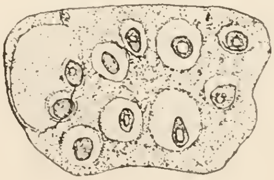

H. J. VAN CLEAVE.

HEMOGLOBIN IN THYONE BRIAREUS LESUEUR.[1]

HAN C. VAN DER HEYDE.[2]

Hemoglobin is by no means rare in lower animals. Though it was originally thought to be characteristic for the vertebrates, it has been found in many other instances. Even in animals as low in the scale of phylogenesis as the worms, we find it in very many forms either in the cœlomic liquid, as in the common earthworm, or in corpuscles (*Glycera, Capitella, Phoronis,* etc.). In Molluscs and Arthropods it is generally found in the free form. It is chiefly found in mud-dwellers, or more in general, in animals living in a medium in which oxygen is scarce. The larva of *Chironomus* is a classical example.

Our knowledge of the occurrence of respiratory pigments in Echinoderms is very incomplete. In one case, in the brittle star, *Ophiactis virens,* hemoglobin has been found. Other statements, *e.g.,* the assumption of a respiratory function of the so-called "echinochrome (Mac Munn. 1885.)" in *Echinus* and *Strongylocentrotus,* are to be accepted with some scepticism. Winterstein, *e.g.,* could show that a solution of echinochrome did not take up any more oxygen than the same amount of sea water. Other data concerning respiratory pigments in Echinoderms have not come to the writer's knowledge.

In studying the digestion of the Echinoderms, among which *Thyone briareus,* a dendrochirote Holothurian common around Woods Hole, the writer was struck by the brilliant red color of the Polian vesicles every time he dissected a specimen. Curiosity drove him to examine this color a little more closely. The color appeared to be due to the contents; it "flows away," when the vesicle is cut. It could be centrifuged off and a microscopical

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[1] From the Marine Biological Laboratory, Woods Hole, Mass.

Received for publication August 30, 1921.

[2] The writer wants to express his indebtedness to Dr. Leo Loeb and Dr. Samuel E. Pond for the loan of microscope and spectroscope and to Dr. Hiroshi Oshima for morphological information.

examination of the fluid showed that it was bound to corpuscles.

These corpuscles are, as far as their appearance under the microscope is concerned, very much like mammalian blood corpuscles; they are however not biconcave discs, but seem to be perfectly round. Their color is the same as that of mammalian corpuscles. They do not have any active movements as far as could be observed, and under the microscope they have the appearance of perfectly round discs. A nucleus is clearly visible in most cases; the protoplasm has a granular structure and a cell membrane gives a perfectly clear "outline."

The question arose as to what the function of these corpuscles was. The Polian vesicle forms a part of the water vascular system. In this way they stand on one hand in connection with the ambulacral feet, which are found all over the outside of the body and by means of which the animal moves. This species is radially symmetrical and does not have the so-called bivium. Inside the muscular body wall these podia are represented by their ampullæ and, remarkably enough, all these little ampullæ can be seen on dissection to contain the same colored material. On the other hand, the Polian vesicle, through the water vascular system, communicates freely with the cœlomic fluid. The stone-canal stiffened by carbonate of lime, which in other Echinoderms and in larval Holothurians communicates with the sea water, forms here so-called "internal madreporites," one or even five, as in *Holothuria tubulosa*. This stone-canal is derived from the larval axial sinus, whereas the original pore-canal and external madreporite disappear. Furthermore the water vascular system stands in connection with the tentacles, the "feelers," which by their active movements may assist greatly in respiration.

The so-called blood system of the Holothurians consists of a blood ring surrounding the œsophagus and situated inside of the water vascular ring. This system forms a plexus around the gut and water lungs, a dorsal "vessel," and probably stands in connection with the cœlomic fluid.

The water lungs are considered to be organs of respiration. A rhythmical contraction of the cloacal wall can be seen, resulting in in- and outflow of water into the surrounding sea water.

The question arose as to what the nature of the red pigment in the corpuscles of the Polian vesicle was.

The following evidence will serve to show that true hemoglobin is present in these corpuscles:

1. A small quantity of the fluid was dried on a slide, heated a few times under cover-glass with acetic acid till bubbles appeared, then cooled. Blue-black prismatic crystals could be seen, which though not absolutely congruent with the figures of text-books of biochemistry, doubtlessly were hemin crystals.

2. Centrifuged and "laked" "blood" showed the typical absorption spectrum of oxyhemoglobin—one broad band in concentrated solution, two on dilution, one of which can be easily identified by means of the D—sodium—line.

3. On reduction with Stokes's reagent the single band of hemoglobin could be seen. After continued vigorous shaking, the double band of oxyhemoglobin could again be produced.

4. Material treated with KCN and kept in the thirty-seven-degree incubator for some time, became orange-yellow and showed the absorption band in the green which characterises cyanhemoglobin.

5. A small quantity of the material gave a positive Prussian blue test for iron.

6. To a solution of one or two drops of 1 per cent. benzidine in a large quantity of hydrogen peroxide a few drops of the "blood" were added. A dark blue color developed at once and a vigorous foaming of oxygen could be seen (peroxidase action).

From this evidence the writer concluded that hemoglobin is present in these corpuscles. This is very interesting in connection with the fact that *Thyone* is a mud-dweller, living in a medium in which oxygen is scarce. Though the controversy between Krogh and other investigators, *e.g.,* Jordan, as to whether hemoglobin actually functions as an oxygen *store,* has not yet been settled, it is beyond doubt, that the presence of a respiratory pigment is a great help in the struggle for oxygen.

In how far the Polian vesicle acts as "mover" of the corpuscles, as a kind of primitive heart, could not be investigated more closely. It is strongly contractile however and is generally supposed to control the hydraulic pressure in the water vascular system. No rhythmical contraction can be observed.

The same corpuscles can be seen in a preparation of the abovementioned water lungs. This makes their respiratory function still more probable. On the other hand the evidence seems to show that also the podia and the tentacles play an important rôle in the respiration of *Thyone,* and that two systems of organs seem to be concerned with one function.

Summary.

Hemoglobin is found, bound to corpuscles, in the Polian vesicle(s), the ampullæ and the wall of the water lungs of *Thyone briareus,* a dendrochirote Holothurian, common around Woods Hole. This fact is interesting in connection with the fact that *Thyone* is a mud-dweller.

Vol. XLII *March, 1922* *No. 3*

BIOLOGICAL BULLETIN

THE INVERTEBRATE COURSE IN THE MARINE BIOLOGICAL LABORATORY.

A CONTRIBUTION TO THE TECHNIQUE OF TEACHING ZOÖLOGY.

W. C. ALLEE,

Marine Biological Laboratory.

In the thirty-four years of its existence, the course in invertebrate zoölogy, given each summer in the Marine Biological Laboratory at Woods Hole, has undergone considerable evolution. It was founded at the first session of the laboratory in 1888. In the first report of the director, Professor C. O. Whitman, the course is outlined thus:

" The work of instruction, conducted by Professor Van Vleck, was confined chiefly to the study of invertebrate forms, such as the sponges, hydroids, ctenaphors, worms, starfishes, sea urchins, lobsters, corals, etc. An excellent series of mounted preparations added much to the value of instruction. Considerable attention was given to histological technique, and a large amount of valuable material for use in the teaching was collected by each member of the class."

Since that time the course has been in the charge of J. S. Kingsley, specializing in comparative anatomy; H. C. Bumpus, marine zoölogy; James I. Peck, morphology; Ulric Dahlgren, histology; G. A. Drew, biology of molluscs; W. C. Curtis, morphology and regeneration; Caswell Grave, morphology and embryology; and W. C. Allee, ecology and general physiology. Several of these men had served a preliminary term as instructor in the course before taking charge, and this experience made for continuity in its development.

In the earlier years, many of the lectures were given by investigators, not connected with the teaching staff. The whole field of zoölogy might be considered in this one course, which was designed particularly to meet the need of teachers. In 1893 the work in embryology was taken over in a separate course under the direction of Professor C. O. Whitman, in 1899 Professor Jacques Loeb first offered a special course in comparative physiology, and in 1919 Professor Gary N. Calkins organized the course in protozoölogy; but elementary aspects of these special courses are still retained in the invertebrate course, wherever they are found useful in the study of the subject.

The present type of organization has been in existence since its initiation twenty years ago, in 1901, by Dr. Drew. The conduct of the course as described then by Caswell Grave[1] has seen no radical change, although there has been a gradual evolution away from histological studies towards observations on living animals, in keeping with the zoölogical tendencies of the times.

The instructor in charge of the course bears full responsibility for the selection of the staff, except that the number of staff members is fixed by the laboratory management; there are at present eight instructors and an assistant who acts as preparator and sees to the supply table. The staff is selected from younger instructors or exceptional graduate students and is chosen for teaching as well as research ability, not to mention the physical strength and energy necessary for a long day's teaching and strenuous field trips. An effort is always made to bring these instructors from universities and colleges in different parts of the country in order to have the various types of training represented. The unusual teaching experience is the principal payment for the instructor's labors, although the stipend he receives, together with the accompanying research facilities, is frequently the factor that makes his presence at the laboratory possible.

The work of the staff is divided in open staff meeting with the instructor in charge as chairman, and the general conduct of the course is there discussed and planned. Each member chooses or is assigned some group or groups of animals with which he is especially familiar, or in which he is particularly interested and wishes to work up in the necessary detail. Not only does he familiarize himself with the forms to be used in the laboratory and with the general literature of the group, but he also spends time studying the representatives found near Woods Hole so as to be able to identify them in the field. He goes through the special literature in order that he may be able to give intelligent information on the points of unusual interest connected with the phylum, particularly the research problems in which it has played or is playing a part.

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[1] "Course of Study in Invertebrate Zoölogy in the Marine Biological Laboratory at Woods Hole," *Journ. App. Microscopy*, 10, 1482–6.

The instructor in charge of the course determines who shall be admitted to the course under the rules of the laboratory, supervises field work and has general oversight of the organization. He takes his regular turn in lecture and laboratory along with the other instructors. These are in turn individually responsible for the organization and presentation of their particular groups. Each instructor meets with the men who are to assist him, goes over in detail the work planned, and for the time being has complete charge of instruction. He is aided by "experience" cards which set forth in some detail the results of past methods, but he is not bound by tradition.

The course ends on the second Tuesday in August and begins six weeks earlier. This gives a total of thirty-six working days which are divided as follows:

Course lectures, 29.

Special lectures, two by staff members and a varying number of invited lectures from Laboratory investigators.

Laboratory periods, about 56. This number varies with the number of mid-week field trips taken.

Field excursions, about nine.

Holiday, the Fourth of July.

The formal day begins at nine with an hour's lecture, usually on the group to be studied in the laboratory period following. Although it is difficult to generalize about the lecture methods, it is common with the larger phyla to present schematically an ideal representative of a given group, trace its phylogenetic development, describe in detail special structures, or characteristic physiological activities, and give the differentiations within the group and their relation to the environment in which they are found.

Some attention is directed to important principles that are especially well illustrated in that phylum; relatively little time goes to details of classification.

Blackboard drawings, chart and models are employed in the lecture. In general, we find that students will follow a blackboard drawing made before their eyes, with more interest and understanding, in spite of imperfections in execution, than a description illustrated by the most carefully drawn chart. These drawings are diagrammatic and capable of rapid copying by the class, in preference to exact and detailed reproductions.

Supervised laboratory study follows the lecture for two hours in the morning and again from two o'clock till four in the afternoon; normally three instructors are kept busy, though more may be called in on special occasions; as in the examination of plankton. The instructor in charge usually stays on an hour or more later with the students who wish aid in studying their material more fully. The laboratory is open at all hours, and it is unusual to find it empty between six in the morning and eleven at night. Special assistance is given on request whenever an instructor can be reached.

The students who take this work are required to have had at least one year's laboratory work in college biology and to present a recommendation from their college instructor. The class is limited to 55, and in recent years there has always been more than this number of applications. Qualified students from colleges which regularly support the Marine Biological Laboratory are admitted first; after these are served admission depends on priority of application and on training. The class is drawn mostly from institutions over the eastern half of the United States from Maine to Nebraska and from New Orleans to Minnesota. At least thirty colleges are represented by one or more students each summer. A few apply who present only the minimum requirement, but over half of the class has been graduated from college.

Teachers and graduate students are about equal in number among the college graduates. Older people who desire the course as a matter of intellectual curiosity are always present and welcome. Admission is not knowingly granted to students who use the course as a convenience to work off college conditions.

No text is required, although students are advised to keep at hand some standard reference book, such as Parker and Haswell's "Text-book of Zoölogy," or Lang's "Comparative Anatomy." Pratt's "Manual of Common Invertebrate Animals" is constantly used with the field work. Drew's "Laboratory Manual of Invertebrate Zoölogy," which was originally developed for this course, supplemented by additional mimeographed directions, made out by the instructors from year to year, form the guide for the laboratory work. The Laboratory library is open to the students and they are constantly given references to the literature, but they are more interested in studying the animals at first hand and read comparatively little.

Typically, the subject of Invertebrate Zoölogy is divided on this plan:

Protozoa: Two lectures and laboratory days, followed by a morning spent in collecting fresh-water protozoa from neighboring ponds and an afternoon's work in identifying the forms collected. The laboratory is a compromise between the usual type study and a requirement of a certain number of recognizable drawings. In 1920, for example, *Arcella* was studied the first half day; slides mounted a half hour before use showed active movement. *Ameba, Actinophrys,* and *Actinosphærium* were also demonstrated. Almost every one has studied *Paramecium,* but by feeding the animals with hay bacteria stained with neutral red, the student, in the course of watching the feeding activity and the subsequent change of the food vacuole from acid to alkaline, unconsciously reviews many points of former observation, to his considerable advantage. Hypotrichs are always in reserve for this work.

The second morning was occupied with *Euglena* and *Volvox,* with *Ophridium,* a green colonial vorticilid, and the gregarine, *Schizocystis,* from *Phascolosoma,* in reserve. The afternoon was spent on the flagellates parasitic in termites.[1] These form an easily obtainable and fascinating group and it was hard to divert

[1] Leidy, Jos., "Parasites of the Termites," *Jour. Acad. Sci. Phila.,* VIII., pp. 425–447 and plates, 1881. Porter, J. F., *Bull. Mus. Çomp. Zoöl.,* XXXIII., 1897. Kofoid and Swezy, Univ. of Calif. Pub. Zoöl., XX., 1919.

the students' attention to *Ephelota* and *Acineta,* the Suctorians found attached to old colonies of *Obelia.*

In the identification of protozoa found in the field, it has been found desirable to ask the student to classify as far as possible; after the order, identification is given or checked by the instructor.

Porifera.—One day, lecture and laboratory. In addition to the more usual study of *Leucosolenia* and *Grantia,* students perform H. V. Wilson's experiment[1] on the dissociation of *Microcione* cells. After squeezing these through miller's bolting cloth, identifying the cells and watching early stages of the plasmodium formation, the slides are put out in a live car under the wharf and taken in for inspection at the end of the course almost six weeks later. Living *Grantia* are sectioned and choanocytes and flagella studied in the normal condition. In order to make this successful the material is not exposed to the air even momentarily until it is sectioned, and then for as short time as possible; the *Grantia* is freshly collected and the razors very sharp.

Cœlenterata.—Four days, lecture and laboratory. In 1919 and 1920 the work on Cœlenterata was begun by starting experiments on regeneration in *Tubularia.* The attention of the class had previously been called to the recent paper of Garcia-Banus and the conflict between that report and the commonly accepted ideas on *Tubularia* regeneration.[2] Data were collected each day under the personal supervision of some staff member who inspected each piece of *Tubularia* and final results were tabulated on the blackboard. The results for 1919 are quoted by Hyman[3] (p. 358) and were supported by the data of 1920 and both confirm the results obtained by Child[4] and Hyman rather than those of Garcia-Banus. Needless to say, class interest in this experiment was intense, particularly among the more advanced students.

The work on the Hydrozoa includes time spent on the structure of *Tubularia* and *Obelia*. These are normally fruiting at the time studied. A morning in which the student sees fruiting *Tubularia* and watches *Actinulæ* emerge and crawl around, added to an afternoon studying *Obelia* which are shedding *Eucope,* makes a day's work that the instructors, at least, never forget. To insure a plentiful supply of *Eucope* or *Actinulæ,* the material is brought into the laboratory a few hours before being studied and allowed to stand in a shallow dish in fresh sea water. The *Eucope* come out more readily if placed in the sun and are to be found on the sunny side of the dish.

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[1] Wilson, H. V., "Development of Sponges from Dissociated Tissue Cells," *Bull. U. S. Bureau of Fisheries,* 30, 1910.

[2] "Is the Theory of Axial Gradient in the Regeneration of *Tubularia* Supported by Facts?" *Jour. Exp. Zoöl.,* 26, pp. 265–275.

[3] "The Axial Gradients in Hydrozoa. III. Experiments on the Gradient of *Tubularia,*" BIOL. BULL., 38, p. 358.

[4] "An Analysis of Form Regulation in *Tubularia,*" *Arch. f. Entw'lungsmech.,* 24, pp. 1–28.

Fruiting *Eudendrium, Clava, Hydractinia, Schizotricha* and *Sertularia* are also available for observation and study. In 1921 following a mild winter and an early spring, we could obtain fruiting *Bougainvillia, Campanularia* and *Pennaria. Gonionemus* is studied alive in some detail and living *Physalia* is usually on hand at some time in the six weeks.

In *Hydractinia* the striking *dactylozooids* are readily demonstrated by removing the hermit crab on whose foster shell they grow and stimulating one edge of the colony by scratching with a needle. Under a low-power lens the wave of stimulus can be followed across the colony as the *dactylozooids* strike. These "persons" of the colony are more abundant near the mouth of the shell than elsewhere. In *Gonionemus,* the lithocysts are readily studied by cutting a V-shaped piece from the margin and mounting aboral side up under the high power.

The work on the development of *Aurelia* is on preserved and stained material, although there are usually living *Aurelia* in the laboratory, and sometimes enough of it to supply the class. The canal system is here best demonstrated by injecting it with air, which is easily done even in preserved specimens.

With *Metridium* a half day is spent in repeating Parker's[1] experiments on feeding reactions and an opportunity is given for dissecting recently preserved specmens that have not yet lost all their color. Prepared sections showing relations of the mesenteries are present for special study and living and preserved *Astrangia* is at hand for those who have done the other work. The last half of the day of the Cœlenterate group is spent on *Pleurobrachia* which Mr. G. M. Gray preserves in life-like transparency. These are studied in the original preservative, rather than in water.

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[1] "The Reactions of *Metridium* to Food and Other Substances," *Bull. Mus. Comp. Zoöl. Harvard,* 29, 1896.

Platyhelminthes.—Three days, lecture and laboratory. This study begins with locomotion and feeding reactions of fresh-water planaria, and regeneration experiments are started. The animals are kept in pond water. We introduce the class to living *Syncœlidium* or *Bdelloura* from the gill books of *Limulus*. These have lost their pigment and the structure even to flame cells, is visible. Cross sections have been used of late years to give a better demonstration of the relation of the proboscis to other structures. The Cestodes are studied with living *Crossobothrium* when sand shark spiral valves are available; if these are not to be had, *Rhyncobothrium* and *Calliobothrium*[1] from the spiral valve of the dogfish are substituted. Flame cells are readily seen in the scolex of any of these. The encysted scolex of *Otobothrium*[2] found in the muscles along the vertebrae of the butterfish gives an example of the everted pleurocerous stage characteristic of the Tetrabothridia. In 1921 a study of embryonic stages of *Rhyncobothrium* was added to supplement the work on the encysted scolex of *Otobothrium*. The following stages were studied: (1) The freshly laid eggs; (2) eggs in which the hexacanth embryos were developed; (3) emergence of embryos from egg case; (4) free swimming hexacanth embryos with ciliated embryophore; (5) embryo from which the embryophore has been torn off. Directions for obtaining this material as worked out by Dr. Bowen are given in the appendix (1).

A half day is spent on *Tetrastema,* the little nemertean from pilings. These are secured by allowing scrapings taken about 6 A.M. to stand for three hours in fairly deep glass jars filled with sea water; the nemerteans can be picked up with a pipette and a lighted candle held by the jar just below the water level makes them more easily recognized. Scrapings spoil if allowed to stand over night. If time permits a half day is also spent on the common rotifers since no other place is available for this neglected group.

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[1] Linton, E., "Notes on the Entozoa of Marine Fishes of New England," U. S. Comm. of Fish and Fisheries, Comm. Report, 1886 and 1887.

[2] Linton, E., "A Cestode Parasite in the Flesh of the Butterfish," *Bull. Bur. of Fisheries,* 26, 1906.

Echinoderma.—Four days, lectures and laboratory. At present this work is introduced by a day's study of maturation, fertilization, cleavage and development stages of the starfish egg, with sea-urchin and sand-dollar material in reserve in case of need. Approximate schedules for different stages in the development of *Asterias, Arbacia* and *Echinarachnias,* as worked out by Miss Christianna Smith, are given in the appendix (II.). Preparation for this day's work takes much time and careful cooperation with Mr. Gray in the matter of securing plenty of ripe starfish, since this is near the end of their breeding season.

Work on the adult starfish begins with a study of the activities of the living animal, which includes an annual repetition of Cole's experiments on righting reactions and locomotion;[1] in spite of diffuse lighting and class technique, these have on the whole supported Cole's contention that there is evidence of physiological asymmetry in the starfish. The free cells in the cœlomic fluid are also studied. We obtain these easily by hanging the starfish up by one arm, catching a drop of the fluid as it escapes and examining immediately before aggregation takes place. Circulation in the cœlomic fluid and the dermal branchiæ is demonstrated by means of carmine injection.

The sea-urchin takes more than a day for dissection even though little time is spent on the arrangement of plates in the test. Aristotle's lantern is studied in detail and the genital rachis in *Strongylocentrotus* is dissected. For this work specimens preserved in alcohol are used. The dissection is made by picking off the skeleton in the equatorial region, leaving one ray intact. The exposure is gradually extended to the dorsal region, which is held up as long as desired by the part of the shell still intact. This gives better results than the classical method of dissection.

Ophioderma and *Leptosynapta* are present in reserve for those who have done the regular work. The latter are kept from the time they are collected in separate bottles placed in a larger dish of running sea-water to prevent fragmentation. We usually study the swimming motions of *Ophioderma*.[1]

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[1] "Experiments on Coördination and Righting in the Starfish," BIOL. BULL., 24, 1913. "Direction of Locomotion in the Starfish," *Jour. Exp. Zoöl.,* 14, 1913.

Living representatives of all available echinoderms are kept in aquaria in the center of the laboratory. Some of these contain starfish and mussels and give the starfish an opportunity to demonstrate their feeding methods. *Thyone* are allowed to bury themselves in muck and show their usual feeding reactions and method of respiration.

The last half day is spent on a dissection of narcotized *Thyone*. For this work freshly collected specimens are best. These are injected about an hour before use with about 15 cc. of chloretone solution recently saturated when hot. After a short time the tentacles can be forced out by gentle manipulation and the narcotization holds during the dissection in sea water. It is essential that the chloretone solution should be as strong as possible. There is a great difference between these narcotized animals and the usual formalin faded specimens.

Annelida.—Three days, lecture and laboratory. *Nereis virens* living, preserved, or narcotized in alcoholic sea water, forms the entering wedge for this group. Cleared transverse sections of whole segments are also used to show general relations and especially the parapodia. *Arenicola,* freshly narcotized, are dissected for the blood system and for the nephridia, following directions based on Ashworth's monograph.[2] Sometimes we dissect *Amphitrite* (see appendix III.) and a large collection of other worms is present in the laboratory. Usually *Lepidonotus* is studied for external features, and of recent years a half day has been spent in classifying local annelids, using Pratt's key. Phosphorescence can be successfully demonstrated in *Chætopterus* by taking the animals to the dark room at night, and the class collects swarming *Nereis limbata* when moon and weather permit. The last half day is normally spent in a dissection of the common sipunculid, *Phascolosoma,* freshly narcotized in alcoholic sea water.

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[1] Grave, Caswell, "*Ophiura* (*Ophioderma*) *brevispina*," Mem. Biol. Lab. Johns Hopkins Univ., 4, 1900.

[2] Ashworth, J. H., Liverpool Marine Biology Committee Memoirs, XI., 1904.

This laboratory work has sometimes been introduced by a study of the 48-hour *Hydroides* trochophores. The primitive structures can be easily made out. These are followed by 5–7 day larvæ showing the greater development of the tail region.

Bryozoa.—One day, lecture and laboratory. Living *Bugula* furnishes the main attraction, and some time is spent on the encrusting forms.

Crustacea.—Three days, lecture and laboratory. The treatment of this phylum has undergone more changes than that of any other group in my eight years on the course. At one time a week was spent in detailed study of their homologies.

We give one afternoon of the three days now allotted them to studying tow, which contains a large number of larval crustacean stages at this season of the year, but is examined for other animals as well.

The lobster is the introductory animal, with the blue crab as alternate for those who have already dissected the lobster or the crayfish carefully. These are injected and hardened (see appendix IV.) in formalin for two weeks before using. One day is given to this dissection, with an extension of time for those who need it. More time could well be spent here but it is better used elsewhere in this crowded course. Some study is made of the usual Entomostraca, such as *Cyclops* and *Argulus* and a half day is spent in working out appendages of Malacostracan forms, which are recorded as shown in appendix V. While on the chart rack the information given in appendix VI. is displayed to aid the students in making comparisons readily among the forms studied.

The work is finished by a half day spent in dissecting *Lepas* with *Balanus* present for comparisons. Among the interesting side lights that have been presented from time to time the following have been especially successful:

Argulus placed in finger bowls with *Fundulus* attach themselves to the fish and change color as the *Fundulus* change from being placed on different backgrounds. This is difficult because of the time required to accumulate enough *Argulus*.

Blood coagulation is shown in *Oniscus* by snipping off the filamentous tip of the antennæ. A drop of blood is caught on the coverslip and by staining in methyl green or aqueous iodine, a cell-agglutinating type of blood-clotting may be observed.[1]

Autotomy is usually demonstrated in *Uca*, the fiddler crab, by crushing the distal end of one or more of the legs with a pair of forceps. Chromatophore activity is well shown in *Palæmonetes.*

Limulus.—One day, lecture and laboratory. We formerly gave a half day to the external anatomy of *Limulus,* using small specimens. At present in addition to the time on the external anatomy and feeding experiments, a half day that usually stretches well on into the night is spent in dissection. *Limulus* is particularly interesting to us as a persistent type, because of its use in phylogenetic theory and because it is a characteristic and common large invertebrate of Woods Hole. The dissection directions have been worked out in the main by Dr. R. H. Bowen and are given in full as Appendix VII, since I know of no other directions in print.

Mollusca.—Five days laboratory, four lectures. This group is introduced by work on *Chætopleura,* which may be mounted on glass slides if put under water, and may then be readily studied on both dorsal and ventral sides. The principal study of the group centers about *Venus* (or *Unio*), *Busycon* and *Loligo.* Usually one instructor is in charge of the earlier part of the work and another has the Cephalopoda.

In the Pelecypoda, comparative studies are made of *Pecten, Mytilus, Mya, Ensis* and *Ostrea,* with a drawing of one form; and living and preserved slides are studied in *Venus, Mytilus* and *Ostrea.* The gill current can best be seen in the latter on account of the large large size of the palps on which ciliary currents can be seen. In the opening dissection, the blood system shows much better if injected through the ventricles.

The work in this group also includes study in locomotion of *Ensis, Yoldia,* and *Solemya,* and egg laying, fertilization and cleavage in *Cumingia.*

The gasteropod work is limited to *Busycon* (*Sycotypus, Fulgur*). These are killed and injected a week before using in order that they may be hardened sufficiently to get rid of slime without being too hard for satisfactory dissection. One and a half days should be spent on this dissection, although the familiar device of allowing half the class to work out and demonstrate the circulatory system while the others reciprocate with the nervous system, disposes of the dissection in a day.

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[1] Tait, *Jour. Mar. Biol. Assn.*, 1911.

Cephalopod study is limited to the common squid, *Loligo.* Living specimens are in the students' aquaria and in the central tanks through the week; egg laying frequently occurs. The most spectacular demonstration of the course comes when the living squid are injected through the buccal sinus for venous system and through the heart for arterial, and studied immediately in salt water. Almost the whole system can be made out without cutting. The better prepared students make the complete dissection of the spermatophoric organ, for which directions are found in the third additon of Drew's Manual. It is obvious that in this work, as on *Busycon,* there is not time for finished dissections and drawings, but it is surprising how much good work can be done in the concentrated effort of a long day, especially if drawings of external features be largely omitted.

Chordata.—Two days, lecture and laboratory. Chordate studies are limited to the Hemi- and Urochordata. The work begins with a study of external features of *Dolichoglossus.* These may be collected Saturday afternoon and kept until Monday in good condition if placed in separate bottles over which a good supply of water flows. The remainder of the first day is spent largely on *Molgula,* living and preserved.

Perophora is used to demonstrate the circultion and the modification found in a stolon type. Young *Botryllus,* that are still transparent, are also present in the laboratory in reserve. *Amarœcium* tadpoles, alive and stained, take up the last morning of the course. The last afternoon is spent in working out some form, such as the adult *Amarœcium,* with no help other than that to be found in texts and laboratory guides. The exercise is not an examination; it counts no more in our estimation of the ability of students than that done on any other half day. It gives the student an opportunity to orient himself under conditions less favorable to rapid work than those that prevailed in the preceding part of the course; and it holds the attention of the class completely even past the official end of laboratory work.

No attempt is made to put the entire class through a fixed schedule of laboratory work; even where everyone is working on the same material, as much as possible of the method of attack and particularly of the method of recording observations is left to the individual. Students who desire space in the laboratory and access to laboratory material but who have their own plan of work they desire to follow, are welcome as a matter of course.

Field Work.—The field work is one of the most important aspects of the entire course; even the anatomy cannot be clearly understood until the animal has been seen in its native haunts. Gross collecting methods are avoided as far as possible and the student is urged to observe carefully while collecting where the animals are found and what they are doing.

The field schedule for 1921 follows:

July 2, Saturday:
  Collecting Protozoa 10:00–12:30.
  Examination of material in laboratory, 2:00–4:00.
July 6, Wednesday:
  Crane's Wharf. Start 2:00; low tide, 3:20; return about 4:00.
July 9, Saturday:
  Vineyard Haven Wharf. Start 8:45; low tide 3:20; return about 2:00.
  Examination of collection in laboratory, 2:00–3:00.
July 16, Saturday:
  Hadley Harbor (Northeast Gutter Flats). Start 10:00; low tide 11:55; return about 3:30.
July 20, Wednesday:
  Hadley Harbor (Gutter Rocks). Start 1:45; low tide, 2:23; return about 4:15.
July 23, Saturday:
  Dredging, Vineyard Sound.
  Section 1, teams 1–4 start 10:00; return about 12:00.
  Section 2, teams 5–8 start 2:00; return about 4:00.
July 28, Thursday:
  Study of "Tow" in laboratory, 2:00–4:00.

July 30, Saturday:
North Falmouth Flats. Start 8:45; low tide, 9:35; return about 3:00.
August 4, Thursday:
Kettle Cove, Rocks and Flats. Start 1:45; low tide, 2:33; return about 5:00.
August 6, Saturday:
Picnic. (1921, Penikese.)

Practically the same field organization has been used for the last eight years. The class is divided into as many teams as there are instructors; the team has the same members throughout the course, but the instructors rotate so that each team has the benefit of three different instructors in the field. In each team one student is recorder for the day; others have various assignments depending on the trip. If this be to a flat, two have shovels, one a bucket and crystallization dish, one a *Cumingia* sieve, and the rest carry collecting kits with bottles and jars, forceps and lenses. All are mentally and physically prepared to get wet and dirty.

The Protozoa field trip follows the Protozoa work in the laboratory. While the class is studying Cœlenterates and sponges, two trips are made to wharf piles. Collecting is done from small boats, and glass-bottomed buckets and scrape nets are an important part of the equipment. The trips to flats and rock gutters, and the dredging both depend for their sequence on the tides. It is a general rule never to take a trip in the morning and attempt regular class work in the afternoon. We have found that the students are too tired or diverted to settle down to class dissection. They are interested in working over the specimens collected on the trip and this informal study has become more common of recent years.

At the beginning of the course the student is supplied with a check list of the animals taken by the class in the last ten years. It is suggested but not required, that these be made the basis of class records of experience for the season, so that the student obtains a permanent record of the habitat distribution found by the class collecting that year.

The recorders are furnished with a board bearing a list of the animals expected from the habitats in the locality to be studied. This is arranged so that an animal may readily be recorded from all the habitats in which it may be found. So far as possible the instructor attempts to teach the students in the field classification down to genus. Species identification is given the student only when of special interest or, in the case of the *Nassas* and *Littorinis,* necessary. The instructor calls attention to interesting facts in the distribution, and other points calculated to make the student more familiar with the animal under consideration. Each instructor is supposed to have a wide knowledge of the group on which he lectures, and doubtful specimens are referred to him for identification. Animals that cannot be named in the field go into the "question mark" bottle for later reference.

On returning to the laboratory a complete list of all the animals found by the class is posted on the board. The list in Appendix VIII. gives the result of one day's find on the North Falmouth Flats in 1920. The list is entirely typical and shorter than the lists frequently reported. No one team found all of these; the individual teams listed from 50 to 96 species, depending on the ability of the team members, the experience of the instructor and the run of collecting luck. It is on record that twice teams have collected and their instructor identified more than a hundred species in one afternoon; but usually there is no effort made to obtain mere numbers of forms. The emphasis is rather on the characteristic animals in the different habitats studied.

While students may make collections and spend considerable time over detailed classification if they desire to do so, they are not urged in this direction. Neither is there any attempt to have students write up accounts of the field trips; even the keeping of records of the animals found is optional. This is in keeping with the general policy of instruction, that as much as possible must be left to the initiative of the student. Each year there are a few students with especial interest in field studies who devote extra time to them; but it is surprising how well the average student learns to recognize and name characteristic animals with no insistence on the part of his instructors.

After one of the early trips all animals taken are demonstrated in separate finger bowls in order that a named collection of living animals be at hand for closer inspection than is possible in the field; later only the rarer animals are demonstrated. The course possesses a named collection of the common molluscs of the region and is accumulating preserved collections of other groups.

With the exception of the afternoons on Plankton and Protozoa, little attempt is made to identify for class purposes the nearly microscopic animals that may be taken, since it is the purpose of the trips to acquaint the students with the larger, more outstanding members of the local fauna. Doubtless we pass over more species than are recorded. The field collecting has given a number of new records for this well-worked region, has yielded material for one study of regional and seasonal distribution,[1] and is the basis for a study of the littoral ecology of Woods Hole now in preparation.

In earlier days and again for the last eight years a catalogue record of species taken has been kept and is on record as a part of the library of the Laboratory. The person in charge of this compilation is frequently called on to suggest to investigators available material suitable for certain investigations, or the whereabouts of animals not usually collected by the supply department.

Near the end of the course the field work is summarized in an evening lecture on the ecology of the region, illustrated by slides obtained from the American Museum of Natural History and by projections of living animals.

The students have the advantage of the bi-weekly evening lectures maintained by the Laboratory; in addition, special lectures directly related to the work of the course are arranged for the benefit of the class. These are usually given on Saturday morning before the field trip for the day, or in the evening, and they not only convey the information, but introduce the class to some of the interesting personalities among the research group. In 1920 these special lectures were given:

"Some Aspects of Physical Organization of Protoplasm," by Robert Chambers.

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[1] Allee, W. C., "Note on Animal Distribution Following a Hard Winter," Biol. Bull., 36, pp. 96–104.

"Observations on Egg-laying in the Trematode, *Epobdella*," by Edwin Linton.

"The Elementary Nervous System," by G. H. Parker.

"Metamorphosis in the Echinoderms," by Caswell Grave.

"Zoölogical Research," by C. E. McClung.

The Laboratory gives no credit for work done in its courses, nor does it keep formal record books. A student so desiring may receive a certificate stating that the work of the course has been satisfactorily completed and may then cash this in at his college for undergraduate, and in most institutions, for graduate credit (*e.g.*, Chicago, Wisconsin, Cornell). All colleges do not allow the same amount of credit; the usual practice is to give from 5 to 6 semester hours' credit for the work of the course. Any student may have his laboratory notes inspected by the instructor in charge of the group, and all persons asking for certificates must submit their notes for such inspection.

The course outlined is sufficiently different from the ordinary college course, both in its method of presentation and its effect upon students, to arouse constant curiosity as to its essential points of variation. At Woods Hole there are unusual temptations to sight-seeing, boating and bathing, but the attitude of the class is anything but the summer-vacation type that prevails in many summer schools.

The great efficiency and speed attained and held by the class through the six weeks must be ascribed in some degree to the presence of three trained teachers, the instructor in charge and the two assisting members of the staff, who are always at hand in the laboratory when needed; though they give as little oral instruction as possible, so that the student is a more independent worker at the end of the course than he was at the beginning.

I am inclined to think that the intensive work which the students accomplish is due in part to the very speed with which ground is covered. It appeals to the imagination of the student to look back at the end of ten days over the Protozoa, the sponges and cœlenterates, with three field trips extra. Further, he has the advantage of no other study to distract his mind. Through the winter his attention must ordinarily be stretched over three to six diverse subjects; these six weeks at Woods Hole belong to zoölogy alone.

He has before him the constantly shifting panorama of the animal kingdom, and each animal group is presented by a new instructor with a fresh point of view and a different method of procedure. It is an interesting sight to watch the class when fagged near the end of the course, rise to the stimulus presented by an instructor with a new way of looking at things in general and this especial group of animals in particular.

Another potent cause for sustained interest is the excellent supply of living or recently preserved animals which Mr. Gray, of the supply department, keeps in the laboratory. Up to the limit of his working ability, the student will not let this material go to waste.

The intercollegiate character of the class personnel gives stimulus to high-grade work. If undergraduate, the students are usually the pick of the department sending them; if graduate students, they are at least serious-minded. Many of them feel that they are personal representatives of their college and more especially of their home department of zoölogy and they take pride in bringing credit to their training.

Finally, there is not the usual reserve between instructors and instructed. To a great extent the general attitude is that of two groups of people, both recognizing that they are fellow-seekers after information, but one, due to greater experience, obliged to confess complete ignorance less frequently than the other group. Even if he desire to do so, the instructor cannot keep a dogmatic attitude in the face of eight students digging all sorts of animals from the sand and mud and asking questions which a dozen specialists could not truthfully answer without an occasional "I don't know."

From my observation of the effects of this scheme of class work in the Woods Hole course, I believe the following aspects could be carried over with profit into the usual winter zoölogical courses.

1. The introduction of more work with living animals, particularly in the introductory course, and the elimination of much

of the work on formalin specimens. This has been done in the Michigan course as outlined by Shull and his associates, and I believe that the method is applicable in the majority of our laboratories. This requires more work in the presentation of a course, since it is easier as well as cheaper to buy, for example, formalin-preserved earthworms than to keep living ones ready for study at the desired time. It necessitates also an acquaintance with the local animals and their peculiarities of structure and habit, when these are not described in the standard textbooks.

2. Aquaria with living animals should be constantly in the laboratories of appropriate classes even though no work is assigned to be done on them. They will interest our better students in spite of themselves, even in the midst of the crowded winter curricula and outside activities. These aquaria may well include a balanced salt water one.[1]

3. Wherever possible, field work should be included with the work in the first-year course, since it is impossible to understand even the morphology of an animal when seen only in the pickle jar and laboratory environment. Students should not be taken out *en masse,* but in carefully divided groups and the trip should yield definite information which the student will recognize as being an integral part of the course. One section of the year's work in the elementary course, preferably the spring, may well be devoted to field studies which should serve to emphasize many of the problems discussed earlier in the year.

4. A number of approximately equally well-trained instructors should be associated in giving the larger courses. I regard the results from the staff here described as definite proof of the inferiority of the usual method whereby one man, in charge of the course, gives all the lectures and others, poorly trained, often be-

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[1] Salt water may be obtained from the sea shore and inexpensively shipped by freight half way across the continent. Living animals for salt water aquaria, together with plants to balance them, may be secured from Mr. G. M. Gray, of the M. B. L. Supply Department. For best results this material should be forwarded soon after definitely cool weather sets in. Mr. Gray always sends more animals than can well be kept and the hardest task is to kill enough at the beginning so that the others may have a fair chance to live. I have kept such an aquarium running for seven months with no trouble other than keeping the water level constant by adding distilled water.

ginning graduate or advanced undergraduate students, give laboratory instruction. I understand the conditions under which this type of instruction has arisen but it is essentially unsound.

The lectures frequently become a routine re-hashing of bygone compilations on phases of the subject of no particular interest to the lecturer, and often quite unrelated to the laboratory work. This is not true in the fortunate laboratories where the lecturer in the large classes is a brilliant master of his subject matter, the English language and the principles of pedagogy, but it holds for too many courses. It is true that a certain coherence of presentation is gained when all of the subject matter passes through one mind, but personal prejudices are likewise transmitted, however unconsciously. Effective teaching is done only when the student is keenly interested and the shifting of lecturers makes naturally for a stimulus of interest.

In the laboratory work, the advantage of the employment of a group of men all well grounded in the essentials of the subject is obvious. The odd findings of an enterprising student can be capitalized, explained on the spot or their source of explanation suggested, when in the ordinary routine of laboratory work such unknowns must of necessity be disregarded by the undertrained or overworked teacher. I am thoroughly convinced that very seldom should instruction be given in college or university by persons under the rank of instructor, with its present connotations, and that courses should be limited or teaching staffs increased until this is possible.

5. The dominant spirit at Woods Hole is research or preparation for research, and this atmosphere does much to color the work of the Invertebrate Course. I am not yet convinced that teaching of undergraduate students stimulates research, or that an instructor who allows himself to be submerged in research should be trusted in an elementary course; but I am certain that, other conditions being equal, the best teaching is done by men actively engaged in some form of research and in a laboratory where the research purpose prevails.

APPENDIX.

In this appendix are given some more technical directions that we have worked out and that are not readily available lesewhere.

I.

Embryology of Rhynchobothrium: Dr. R. H. Bowen.

Take from the spiral valve of the dogfish the ripe, free, milk-white proglottids of *Rhynchobothrium* and place them in finger bowls of fresh sea water; ten or a dozen may be put in one bowl. Care should be taken not to carry over too much of the digestive slime as it is not feasible to change the water once the eggs are laid. If the proglottids are ripe they discharge the eggs at once in a white cloud. The empty proglottids should be removed and the finger bowl set aside without agitating the water. The eggs soon become dark brown or blackish and stick together and to the bottom of the dish in a solid mass. By keeping the eggs thus bunched their concentration can be controlled when removing them for study. Fresh eggs can be examined at once after laying, but they offer nothing of special interest. After not less than three days and up to periods of five to six days, the hexacanth embryos are completed and ready to emerge from the egg cases. Remove a part of the cluster of eggs by scraping them off and sucking up in a pipette and examine under the microscope. The mechanical disturbance of moving them seems to stimulate the embryos, for it is usually easy to find a number of embryos just emerging from the egg-cases; also free swimming embryos and empty egg-cases. Cultures four days old are generally the best.

II.

Approximate Schedule for Stages in Echinoderm Development Under Mid-July Conditions, from the Experience of Miss Christianna Smith.

1. *Asterias forbesi.*

Stages.	Time after Fertilization.
Polar body formation	45–60 minutes.
Two celled	2–3 hours.
Four to eight celled	3–4 hours.
Sixteen celled	4–5 hours.
Late blastula	7–10 hours.
Early gastrula	18–21 hours.
Late gastrula	24–30 hours.

2. *Arbacia punctulata.*

Two celled	1 hr., 30 minutes.
Four celled	2 hrs., 30 minutes.
Eight celled	2 hrs., 45 minutes.
Sixteen celled	3 hrs., 45 minutes.
Sixty-four celled	4 hrs., 15 minutes.
Early blastula	7 hrs., 15 minutes.
Late blastula	14 hours.
Early gastrula	23 hrs., 30 minutes.
Pluteus	Two days.

3. *Echinarachnius parma.*

Two celled	1 hr., 35 minutes.
Four celled	2 hrs., 45 minutes.
Sixteen celled	3 hrs., 15 minutes.
Sixty-four celled	4 hrs., 15 minutes.
Early blastula	6 hrs., 15 minutes.
Late blastula	10 hours.
Early gastrula	21 hours.
Late gastrula	38 hours.
Pluteus	Two days.

III.

Dissection of Amphitrite ornata: Dr. E. F. Adolph.

From a doped or freshly killed specimen obtain some of the cœlomic fluid by making a small slit in the body wall. What is the color and consistency of this fluid? Examine under the microscope and observe the varieties of cells present and their movements. Some of them are reproductive products.

Pin down the specimen, either fresh or preserved, under sea water with the dorsal or bristle-bearing surface upward. With the scissors cut along the mid-dorsal line, piercing only the body wall. Pin the body wall out flat as you cut from head to tail.

Cœlom.—Are there transverse septa or diaphragms? Note the dorsal and ventral mesenteric strands which hold the digestive organs in place. Cut the dorsal ones. How do the details of internal segmental organization correspond to the external organization?

Circulatory System.—This must be observed before further dissection. Find the large, paired, sub-intestinal vessels. Trace them backward as far as you can and forward into the single gastric vessel which continues into the dorsally situated heart. From the heart the branchial vessels go forward to the three pairs of gills. Find the ventral vessel and note its transverse branches, some of which encircle the digestive organs. You may be able to see also the dorsal, neural, lateral and esophageal vessels, all longitudinal.

Digestive System.—Is the mouth ventral or dorsal? It leads into the straight muscular esophagus. How is the latter held in place? Is there a protrusible pharynx? The esophagus opens into the wide straight stomach. Does it show any segmentation? The winding portion of the alimentary tract is the intestine which continues to the anus. Cut open the stomach and note the character of its walls.

Muscles.—Find the transverse-oblique, longitudinal, setal, and circular muscles. Make a rough diagram of the animal through the stomach region, showing the relative positions of the muscles, stomach, and blood vessels.

Nephridia.—Cut through the oblique muscles in anterior somites and expose the nephridia. Quickly sketch the natural position and shape of one of them. How many external openings or nephridiopores do you find? Put a nephridium from a freshly killed worm under the microscope. How do you think the nephridium functions in the living body? The gonadal glands are small white glands ventral to the esophagus. Ventral to them are the larger and yellowish "stomach glands." The nephridia may serve as gonoducts.

Nervous System.—Displace the digestive organs to one side and find the ventral nerve cord. Has it ganglia? Can you find paired transverse branches? What features of the nervous system are metameric? How far does the ventral nerve cord go posteriorly? Anteriorly try to find the sub-esophageal ganglion, the circum-esophageal connectives and the supra-esophageal ganglion. Where would you expect to find sense organs in *Amphitrite?*

Make a list of the special features of the anatomy in *Amphitrite* that are especially significant in view of its habit of living. Compare the anatomy with that of *Nereis*. Which of the two species do you consider to be the more highly differentiated? Which is better fitted for self-maintainance and self-preservation? Make a table of the functions performed by the body of *Amphitrite* under two headings; those without which the animal or species could not survive, and those which are luxuries. Are any of these functions performed in a way which you consider more efficient by animals you have already studied in this course?

IV.

INJECTION OF BLUE CRAB (CALLINECTES SAPIDUS).

The location of incision to reach the heart is shown in Fig. 1. The injecting syringe should be inserted in horizontal position.

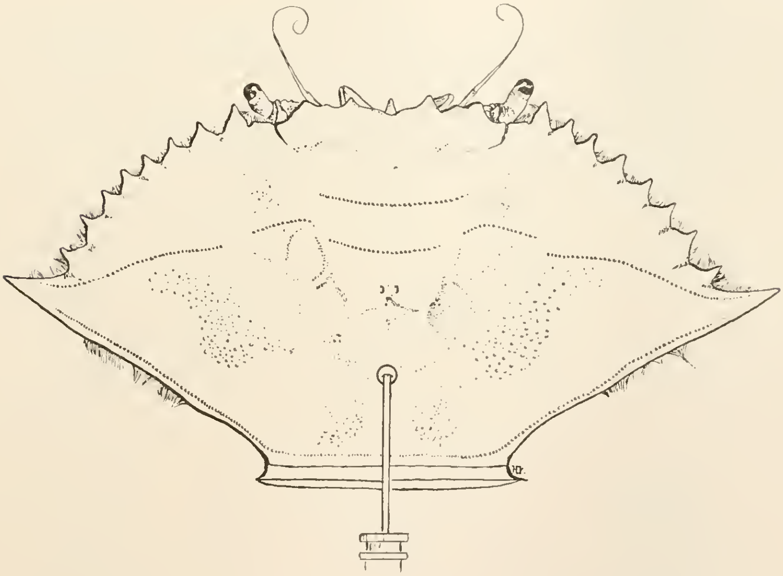

FIG. 1. Dorsal view of the blue crab, showing method of injecting through the heart. Drawn from an injected specimen by Helen Daniels Young.

V.

Table I.

A Comparative Study of Appendages of Common Woods Hole Crustaceans.

Reworked with aid of Mr. J. P. Visscher and Dr. J. A. Dawson from a table made by Dr. G. S. Dodds.

	Lobster.	Blue Crab.	Hermit Crab.	*Hippa.*	*Mysis.*
0	Stalked eye	Stalked eye	Stalked eye	Long stalk	Stalked eye
1	Antennule	Do. small	Do. Short, single	Do. Small, 2 flagella	Antennule
2	Antenna	Do. small	As lobster	Coiled under mouth parts	Antenna large exopod
3	Mandible Palp 3 seg.	As lobster	As lobster	Mandible soft	Mandible small
4	1st Maxilla No endopod	Do. biramous	As lobster	As lobster	1st Max. small
5	2d Maxilla Scaphognathite	As lobster	As lobster	As lobster	2d Maxilla Biram. plumose
6	1st maxilliped biramous, endopod with 2 joints	As lobster	As lobster endopod unjointed	1st maxilliped	Biramous leg
7	2d maxilliped biramous, endopod with 4 joints	As lobster	As lobster	As lobster folded	Biramous leg
8	3d maxilliped biramous, endopod with 5 joints	As lobster flattened	As lobster	3d maxilliped operculate	Biramous leg
9	Large chela	As lobster	As lobster	Digger uniramous	Biramous leg
10	Chelate leg	Non-chelate leg	Do.	Digger uniramous	Biramous leg
11	Chelate leg	Non-chelate leg	Do.	Digger uniramous	Biramous leg
12	Non-chelate leg	Non-chelate leg	Reduced	Digger uniramous	Smaller leg
13	Non-chelate leg	Swimming leg	Leg small	Long, folded	Brood sac from base in female
14	♂ Small modif. ♀ Biram. small	♂ Modified ♀ Biramous	o or left only	♂ o ♀ Small	Swimmeret small
15	Swimmeret ♂ Slightly modif.	♂ Modified ♀ Biram.	Small, left only	♂ Uniram. ♀ Small	Swimmeret small
16	Swimmeret	♂ o ♀ Biram.	Small, left only	♂ o ♀ Small	Swimmeret small
17	Swimmeret	♂ o ♀ Biramous	Small, left only	o	Swimmeret
18	Swimmeret	o	o	o	Swimmeret
19	Uropod Biramous	o	Uropod hooks, left larger	Biramous	Uropod long Lithocyst
20	Telson large	Telson small, pointed	Telson hooked	Do. long, pointed	Do. long, flat

All these have fused cephalothorax.

[1] Tables I. and II. are inserted, although not prepared for publication, because so many teachers of experience have spent time in copying them when given the opportunity.

	Talorchestia.	*Caprella.*	*Oniscus.*	*Argulus.*
0	Non-stalked eyes	Do.	Do.	3 non-stalked eyes
1	Uniramous, short 1st antenna	Do. long	Rudimentary	Minute, 4 segments
2	Uniramous, 2d antenna. ♂ long, ♀ short	Shorter	Well developed	Small
3	Mandible, chitinous teeth and palp	Minute	Mandible	3 and 4 fused in proboscis to form piercing mouth parts
4	1st Maxilla biram. small	Minute	1st Maxilla	
5	2d Maxilla biram. small	Minute	2d Maxilla	Slender
6	1st Maxilliped fused, 2 lobed lower lip	Small	Maxilliped	0
7	Small uniramous hooked	Sub-chelate	1st leg	Suckers
8	Sub-chelate, large in male	Do.	2d leg	Leg-like
9	Uniramous leg	Gill, Brood pouch in female	3d leg	Large leg
10	Uniramous leg	Gill, Brood pouch in female	4th leg	Large leg
11	Uniramous leg pointing backwards	Leg	5th leg	Large leg
12	Uniramous leg pointing backwards	Leg	6th leg	Large leg
13	Uniramous leg pointing backwards	Leg	7th leg	0
14	Swimmeret	Abd. rudimentary	Slender endop. gill exopod, operculum	Unsegmented
15	Swimmeret	Abd. rudimentary	Slender	Unsegmented
16	Swimmeret	Abd. rudimentary	Slender	Unsegmented
17	Jumper large biramous	Abd. rudimentary	Slender	Unsegmented
18	Jumper large biramous	Abd. rudimentary	Slender	Unsegmented
19	Jumper large biramous	Abd. rudimentary	Biramous feelers	Unsegmented
20	———	———	———	———

Fusion

	1st thoracic fused to head	1st and 2d thoracic fused to head	1st thoracic fused to head	3 free thoracic segments

VI.

Table II.

Comparative Morphology and Physiology of Different Groups of Crustacea. Compiled by Dr. Raymond Binford.

	BRANCHIOPODA	OSTRACODA	COPEPODA	CIRRIPEDIA	MALACOSTRACA
Body Segments	Vary, 13-42. Abdomen no appendages. Apus 50 " " on 17 segments.	Indistinct. No limbs on posterior part.	16. Antennules, antennae mandibles, 2 maxillae, 1 maxilliped = 6. 5 with biramous app. 5 abdominal	Indistinct. No abdomen	19. 8 thoracic. 6 abdominal.
Carapace	None, shield or bivalve, attached to only a few segments in front.	Bivalve.	Absent or reduced.	Mantle of several plates Bivalve-shell in larva	Variable. Covers cephalothorax in many.
Tail	Usually a caudal furca	Caudal furca.	Caudal furca.	Caudal furca in most forms.	Telson. Caudal furca in very few.
Eyes	Paired, compound. Nauplius eye usually present.	Compound in a few Myodocopa. Nauplius-eye present in most.	Nauplius-eye No compound eye.	Nauplius-eye on dorsal surface of stomach	Compound on movable stalks.
Antennules	Small, unsegmented, sensory. Clasping organs in some ♂-Cladocera.	Large. Sensory or locomotory. 8 segments. Some less.	Uniramous. 25 segments with exceptions. Swimming or clasping organs	Used for attachment.	Bears 2 flagella.
Antennae	Anostraca claspers. Notostraca absent or vestigial. Concostraca, Cladocera swimming organs.	Large. Chiefly locomotory. Bears spinning gland in Cytheridae.	Usually biramous, swimming organs.	Disappear in adult.	Scale for exopodite in most.
Mandibles	No palp.	Palp large. Labrum. Clasping organ in Sarsiella.	Sometimes keeps biramous structure in adult	Palp rudimentary.	Lacinia mobilis often present.
Maxillae	1st small, lobed. 2nd mostly small. In Cladocera absent	Absent or variously modified.	Present. 2nd sometimes clinging organs	1st present. 2nd united in lower lip.	Both maxillae present
Appendages	Foliaceous. Respiratory bract.	4 or less back of mandibles. Leaf-like or leg-like. Posterior leg-like.	1 uniramous maxilliped Clinging in some. 5 pairs biramous legs.	6 biramous pairs.	Thoracic & abdominal present & distinct
Locomotion	Swimming by foliaceous appendages. Conchostraca, Cladocera by antennae.	Antennae. A few by antennules.	Antennules, antennae & thoracic appendages	Adults sessile.	Flexible abdomen, tail fin walking legs, swimmerettes
Capturing Food	By gnathobases of appendages.	Large mandibles and labrum.	Mandibles. 2nd maxillae.	Thoracic appendages.	Prehensile legs, several mouth parts.
Excretion	Maxillary gland. Larvae have antennal gland.	Glands at bases of ambulatory legs in some. Cypris antennal & maxill. glands	Maxillary gland.	Maxillary glands.	Antennal glands more common.
Reproduction	Sexes separate. No spermatophores. Most sperms spherical. Development in uterus or brood pouch. Nauplius.	Sperms filiform. Some spherical. No spermatophores. Eggs develop in shell, on plants or free in water. Modified nauplius	Spermatophores. Eggs in packets attached at opening of oviduct. Nauplius.	Hermaphrodite. Sperms filiform. Development in mantle cavity. Nauplius with few exceptions.	♀ apertures 6th, ♂ on 8th thoracic segment. Spermatophores.

VII.

Laboratory Work on Limulus: Dr. R. H. Bowen, Dr. C. L. Parmenter and Mr. D. B. Young.

In addition to the work outlined in Drew's manual, the following studies can be readily and satisfactorily made:

I. *Method of Feeding.*—Place a large *Limulus* on its back and wait until it is perfectly quiet. Then place bits of mussel, drops of fluid from a macerated mussel, and bits of *Nereis* on the spiny bases of the legs and note the rôle played by each appendage.

II. *Dissection.*—A longitudinal section of *Limulus* cut just a little to one side of the median plane shows that the various organ systems are arranged in layers. Suspended from the dorsal carapace is the tubular heart surrounded by the pericardium. Below the heart is the digestive tract. This consists of the esophagus, a tube which leads forward to the stomach or proventriculus, which turns upward and backward and opens into the straight intestine. At the junction of the esophagus and proventriculus is a plate of skeletal material, the endocranium, which continues back through the cephalothorax. Below this internal support is the central nervous system. Such a dissection, although useful, does not show the various organs as well as does a dorsal view. Therefore the following method is advised. Students will work in pairs and trace out in succession the vascular, the digestive, and the nervous systems.

1. For dissection use adult, live animals not over 10 to 12 inches across the carapace. The larger ones have a very strong exoskeleton to cut through. Place the animal on its back. Cut off the legs leaving the coxal joint attached to the body. Cut around the carapace as shown by the dotted line in Fig. 2. Use a heavy knife, such as a well-worn oyster knife. Begin at the point marked " 1 " and keep well in from the edge to avoid the strong trabeculæ which pass dorso-ventrally along the border. Then go back to the abdomen and make the cuts marked " 2 " and " 3." After this cut through the interarticular membrane on the dorsal side of the caudal spine.

The spine and ventral piece of exoskeleton may then be lifted up from the dorsal carapace and the cuts " 2 " and " 3 " carried forward allowing the knife to follow the path of least resistance. Do not cut too far from the median line on account of the trabeculæ. Finally separate the ventral leaf with the viscera attached from the dorsal leaf of the exoskeleton which may be thrown away. Use the hand to make the separation. Cut the muscles where necessary with a scalpel but take due care not to injure the pericardium and heart lying along the mid-line. Finally lift out the whole of the visceral mass and place, dorsal side up, in a deep crystallization dish. Continue the dissection under sea water.

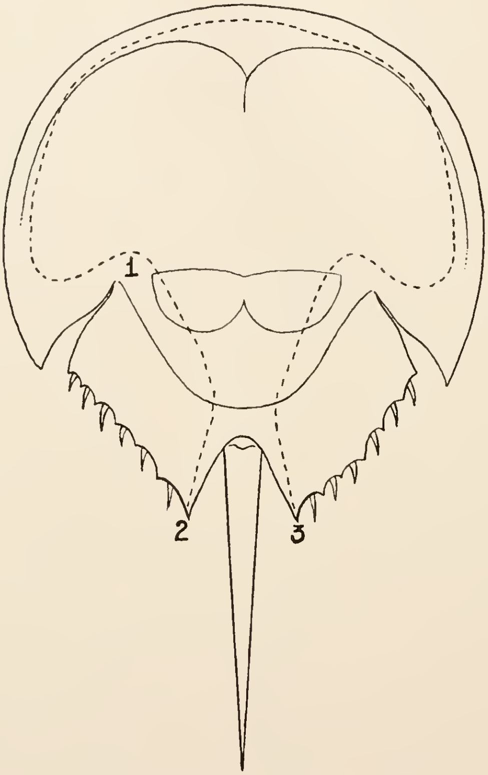

FIG. 2. Diagram of ventral view of *Limulus*, showing location of line of primary incision.

2. The mush which covers the lateral parts of the animal is composed of gonad (orange) and liver (yellowish-green).

3. The heart[1] and its vessels are more or less exposed when the carapace is removed. Carefully free the pericardial area from gonad, etc., preserving if possible the frontal artery, the aortic arches, and the lateral arteries. In some cases the collateral arteries and their fusion to form the superior abdominal artery can be seen. Some of the branchio-cardia canals, which return blood from the gills to the pericardium, can also be identified. Note also the median cardiac nerve and make out as much as possible of the heart itself and its eight pairs of ostia. Then have your dissection demonstrated by an instructor. (The vascular system is not well shown in such fresh material, but the main features can usually be indicated.)

4. Remove the heart exposing the digestive system. Clear up the neighborhood in the same way as for the vascular system. Take care to preserve the large ducts by which the liver communicates with the intestine. The digestive system is a very simple one and will require little or no demonstration by the instructors.

5. Cut the digestive tract near the posterior end and reflect it forward. This exposes the cartilaginous endocranium. The central nervous system lies directly beneath the endocranium, which must first be removed in order to expose the nerve ring. This can best be done by cutting along the median line of the cartilage with scissors, beginning at the anterior end and taking great care not to damage the tissues beneath. Reflect the halves of the endocranium laterally. This exposes the nerve ring and ventral nerve cord. Carefully clear up the area; work the nerves free with fine curved forceps or needles. Take great care to damage the nervous structures themselves as little as possible. Finally work back along the ventral nerve cord and expose the five ganglia; do not lift the cord as the nerves radiating from the ventral surface of the ganglion will thus be completely destroyed. Special

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[1] The circulatory system of young specimens (carapace of three inches) may be injected through the heart by inserting the needle in the median line in the groove between the carapace and abdomen. The entire circulatory system may be followed by removing the dorsal part of the carapace, since the injected vessels may be readily seen through the semi-transparent ventral body wall. Larger specimens may be similarly injected with some advantage in dissecting.

care should be taken in clearing up the area just anterior to the forebrain. Otherwise the delicate olfactory nerves and the nerves to the eyes will be entirely destroyed. Similar care should be taken to preserve the long and delicate nerves which pass from the posterior border of the main nerve ring to the chilaria and operculum.

The nerves arise from the central nervous system in groups of two; one dorsal, called the hæmal, and one ventral, the neural From the nerve ring, eight pairs originate; six large anterior ones and two smaller posterior ones. In addition a median and two lateral olfactory nerves; a median and two lateral eye nerves, arise from the front of the ring. Great care must be used in finding them. The ventral nerve cord has five ganglia with pairs of hæmal and neural nerves from each. The last three sets of nerves join the posterior end of the cord. Find as many of the nerves as you can. An instructor will demonstrate and complete the dissection where possible.

6. Study the arrangement of the coxal glands, the brick-red tubes which lie lateral to the nerve ring. This is the excretory system.

In the absence of an instructor the following papers will be of assistance:

Lankester, E. Ray. Is *Limulus* an Arachnid? Quart. Journ. Micr. Sci., 21, N. S., 1881.

Placed *Limulus* with the Arachnida.

Patten and Redenbaugh. Studies on *Limulus,* I. The Endocranium of *Limulus, Apus* and *Mygale.* Jour. Morph., 16, 1899. Studies on *Limulus,* II. The Nervous System of *Limulus* with Observations upon the General Anatomy. Jour. Morph., 16, 1899.

Morphological account; good figures; direct aid in dissection.

Patten and Hazen. Development of the Coxal Glands, Branchial Cartilage and Genital Ducts of *Limulus polyphemus.* Jour. Morph., 16, 1900.

Patten, Wm. The Evolution of Vertebrates and their Kin. Blakiston, Phila., 1912.

Complete bibliography.

VII.

Table III.

Class List as Posted on the Afternoon of August 8, 1920, on Returning from a Collecting Trip to the North Falmouth Flats.

(Collecting from 3:20 to 6:00; low tide 5:00.)

Porifera
- *Cliona*
- *Microcione*
- *Tethya*

Cœlenterata
- *Edwardsia*
- *Eloactis*
- *Hydractinia*
- *Pennaria*
- *Podocoryne*
- *Obelia commissuralis*
- *Sagartia leucolena*
  - *luciæ*
  - *modesta*
- *Sertularia*
- *Eudendrium album*

Platyhelminthes
- *Cerebratulus*
- *Lineus*
- *Micrura*
- *Stylochus*
- *Syncœlidium*

Nemathelminthes
- *Pontonema*

Echinoderma
- *Autolytus*
- *Arabella*
- *Arenicola*
- *Cirratulus*
- *Clymenella*
- *Diopatra*
- *Enoplobranchus*
- *Glycera* sp.
- *Harmothoe*
- *Hydroides*
- *Lepidonotus*
- *Lumbrineres tenuis*
- *Nereis limbata*
  - *virens*
- *Pectinaria*
- *Phascolosoma*
- *Phyllodoce*
- *Pista*
- *Platynereis*
- *Podarka*
- *Polycirrus*
- *Sabella*
- *Sabellaria*
- *Scoloplos acutus*
  - *fragilis*
  - *robustus*
- *Spirorbis*
- *Sthenelais*
- *Maldane*

Bryozoa
- *Ætea*
- *Bugula*
- *Crisia*
- *Flustrella*
- *Lepralia*
- *Membranipora*
- *Schizoporella*

Arthropoda
- *Amphithoe*
- *Balanus*
- *Callinectes*
- *Caprella*
- *Carcinides*
- *Chiridotea*
- *Crangon*
- *Gammarus*
- *Heteromysis*
- *Idothea baltica*
  - *metallica*
- *Libinia dubia*
  - *emarginata*
- *Limulus*
- *Pagurus pollicaris*
  - *longicarpus*
- *Palæmonetes*
- *Panopeus*
- *Pinnixa*
- *Orchestia*
- *Talorchestia*
- *Ovalipes*
- *Uca*
- *Virbius*
- *Hausterius*
- *Jæra*
- *Ericksonella*

Mollusca
- *Chætopleura*

Pelecypoda
- *Anomia*
- *Cumingia*
- *Ensis*
- *Lævicardium*
- *Macoma*
- *Mactra*
- *Modiolus demissus*
  - *modiolus*
- *Mytilus*
- *Nucula*
- *Pecten*
- *Petricola*
- *Solemya*
- *Tellina*
- *Teredo*
- *Venus*
- *Mya*
- *Ostræa*

Gasteropoda
- *Acmea*
- *Bittium*
- *Busycon*
- *Columbella avara*
  - *lunata*
- *Crepidula convexa*
  - *fornicata*
  - *plana*
- *Lacuna*
- *Littorina litorea*
  - *palliata*
  - *rudis*
- *Nassa obsoleta*
  - *trivittata*
- *Natica duplicata*
  - *heros*
- *Odostomia*
- *Urosalpinx*

Chordata
- *Amarœcium*
- *Botryllus*
- *Dolichoglossus*
- *Molgula*
- *Perophora*
- *Styela*

126 species

CILIARY AND MUSCULAR LOCOMOTION IN THE GASTROPOD GENUS POLINICES.

MANTON COPELAND,

Searles Biological Laboratory, Bowdoin College.

Introduction.

Although gastropod locomotion has often been made a subject of investigation by naturalists and physiologists with the result that the characteristic muscular form of progression is now fairly well understood, it appears that little is known concerning the peculiar and equally interesting activities of cilia which occur on the feet of numerous species of snails, and the part they may play in locomotion, notwithstanding the fact that Quatrefages as early as 1843 believed them to be locomotor in function. A study of pedal cilia in two species of marine gastropods belonging to the genus *Alectrion* led me to conclude not only that locomotion was due to ciliary action, but also that this action, unlike that of ordinary cilia, was either directly or indirectly under the control of the nervous system (Copeland, '19). Recently similar studies on the genus *Polinices* have been carried on at the Marine Biological Laboratory at Woods Hole, the results of which are here recorded. This group appears to contain the largest animals which have been reported as exhibiting ciliary locomotion. The snails also have a muscular form of progression, thus showing a combination of locomotor methods which I believe has not been described for marine gastropods.

Most of the work has been done on *Polinices* (*Neverita*) *duplicata* (Say), although the more northern species *Polinices* (*Euspira*) *heros* (Say), which is closely similar to the preceding, has been studied to some extent. Unless otherwise specified, observations here noted refer to the former species.

The large foot of *Polinices* is differentiated anteriorly into a well-developed propodium which is prolonged over the head region and a part of the shell. This organ, bluntly pointed at the anterior end, is capable of great extension and freedom of movement, and when protruded bears a striking resemblance to the mammalian tongue. The remaining portion of the foot is elliptical in outline and possesses a dorsal area which is reflected over a section of the shell. The ventral pedal surface is covered by ciliated epithelium which is richly supplied with mucus. The dorsal surface of the propodium is also ciliated. The effective strokes of the cilia are directed toward the posterior end of the foot and there is no indication that they are ever reversed.

Ciliary Behavior and Locomotion.

When *Polinices* is placed in a glass dish filled with sea water, it usually exhibits a gliding type of locomotion quite distinct from another form of progression which it sometimes employs involving marked muscular contractions of the foot. The former method may be considered first. A close inspection of the foot as it slides over the substrate fails to reveal any indication of rhythmic pedal waves, all regions of the organ moving at the same speed over a thick layer of mucus. Slight muscular contractions produce a rippling motion along the anterior border of the propodium. The same mode of progression is seen when the snail is moving over a sandy or pebbly substrate, and is essentially like that described for *Alectrion obsoleta* (Parker, '11; Copeland, '19) and *Alectrion trivittata* (Copeland, '19), where locomotion has been shown to be due to cilia beating in mucus which covers the sole of the foot and adheres to the substrate (Copeland, '19). A young *Polinices heros* was also noted moving in a similar manner in an inverted position along a band of mucus laid down on the surface film of the water.

In *Alectrion* it was found that actively beating cilia are characteristic of a moving snail, whereas they are quiescent, or nearly so, when the animal is motionless, as would be expected if the cilia function as locomotor organs. Accordingly *Polinices* was first studied with the object of determining whether the behavior of the pedal cilia was like that recorded for *Alectrion*. An adult snail is too large for successful microscopic examination, but a young individual (*P. heros*) whose foot measured 22 mm. in length was observed while moving over a slide in a small amount of water and the cilia on the thin posterior portion of the foot could be seen beating actively. The same animal was again examined when resting with a partly contracted foot in a culture slide, and at this time all the cilia discernible were either quiescent or showed but slight activity over local areas. The behavior of the pedal cilia of an adult *Polinices* is unquestionably like that of the immature, for when the progression of one is suddenly prevented by holding back the shell, the mucus on the bottom of the foot is driven posteriorly by the beating cilia and objects such as sand grain, pieces of filter paper or even a pin are swept along with it. If now the snail is removed from the water, the propodium may be extended and moved about, at which time the mucus glides over the entire ventral pedal surface and the flicker of beating cilia can be plainly seen by the use of a lens. On the other hand, if the animal is quiescent, either in the water or when held out of it, there is no movement of mucus and in the latter position no indication of ciliary activity is distinguishable.

Polinices placed upside down in a dish of sea water shows a righting reaction which is characteristic. The tongue-like propodium is extended, and the tip directed downward and backward until the ventral anterior margin of the foot is applied to the glass. The attached surface of the propodium is then advanced until the inverted shell lies over the right anterior border of the foot. If the animal does not succeed in applying more of the surface of the foot to the glass, the shell and posterior part of the foot move backward as the propodium progresses, the whole body tending to circle clockwise. If during this activity sand grains are sprinkled over the foot, they are carried posteriorly with the mucus by the beating cilia. No muscular waves are to be seen on the portion of the propodium which is adherent to the glass and locomotion at this time is evidently accomplished by the cilia. On failing to right itself the snail rests with the propodium still in contact with the substrate, or more frequently with the organ withdrawn and held in a somewhat contracted condition with its ventral surface upward. Sand grains now remain on the foot where they are placed and there is no movement of mucus. When, however, the righting activity is resumed the sand and mucus again move down the foot. The righting of the body is finally accomplished after more of the pedal surface is applied to the substrate, particularly that portion along the right side posterior to the propodium, when the rest of the foot and the shell are thrown over to the left. The expanded hind part of the foot shows no muscular movement during the entire reaction.

The preceding observations lead to the conclusion that pedal cilia are beating actively at the time of locomotion, when the foot is functioning as a righting organ, and when it is being moved about as the snail is held out of the water, and that cilia are quiescent, or nearly so, when the animal is at rest either in the water or out of it. The results of certain experiments may now be recorded which in my opinion leave little doubt that these cilia function as organs of progression.

A comparison of the rate of locomotion with the speed at which objects were driven over the pedal surface by beating cilia, or what may be termed the ciliary rate, was obtained in the following way. A snail which was moving around the periphery of a shallow glass dish filled with sea water was stopped by holding back the shell, but grains of sand adhering to the mucus were carried along the under surface of the foot by the active cilia as the animal attempted to continue its course. By the use of a millimeter rule and mirror placed beneath the dish it was possible with a stop-watch to obtain records of the rate of their movement. One of the lateral margins of the foot was slightly curled upward and some of the records were taken as the sand passed over this area; other grains were timed as they moved with the mucus nearer the central and posterior portions of the expanded foot. The average rate of movement over a distance of 5 mm. in ten trials was found to be 2.7 seconds. Immediately after the ciliary rate was obtained the snail was allowed to proceed over the bottom of the dish and ten records of its speed were secured, which showed that it was moving at the average rate of 5 mm. in 2.6 seconds. The remarkably close correspondence between the locomotor and ciliary rates strongly supports the view that progression is accomplished by ciliary action.

Watching *Polinices* glide over the bottom of an aquarium one gains the impression that the animal is too heavy to be propelled merely by the lashing of cilia in a film of mucus spread over the substrate. That such a conclusion is in reality a false one was proved by a few simple tests. As has been pointed out, the pedal cilia are active when the snail is attempting to right itself, and at that time it is possible to determine their driving power by placing objects on the expanded ciliary surface. In this manner it was found that a five-cent piece and good-sized pebbles were readily moved by the beating cilia. Following these preliminary tests, a carrier weighing slightly over half a gram was made by fastening a glass ring on a 22 mm. cover glass. A 20-gram weight was then set on the ring, and the cover glass placed on the upturned middle portion of the foot, which in turn was supported so that the cilia were at about the level of the surface of the water. When the snail began the righting reaction the beating cilia moved the mucus and carrier with its load toward the posterior end of the foot. One of the animals tested in this way weighed between 8 and 9 grams in water and had a pedal ciliated surface with foot expanded of about 54 square cm. Thus between 4 and 5 square cm. of active cilia were able to move not less than 20 grams, whereas during locomotion the task of moving 8 grams was distributed over an area of 50 square cm. The largest specimen of *Polinices duplicata* which I was able to obtain weighed only 36 grams in water and its locomotor surface measured about 132 square cm. Although the resistance of the water to be overcome by the moving animal is a factor to be considered in any detailed calculation of the work required of the locomotor apparatus, the results of these tests leave no doubt as to its adequacy.

Although *Polinices* was once observed to move a short distance clinging to the under side of a glass plate with the propodium unattached to the glass, it is the anterior border of this organ which as a rule holds most tenaciously to the substrate when one attempts to dislodge the snail. Several times I have seen one move up the glass side of an aquarium until it came to the surface of the water, when it turned the propodium downward, or perhaps parallel to the surface, and lifted it from the glass. Soon after relinquishing this anterior hold it slipped to the bottom. Moreover, it is able to cling to the under side of a glass plate by the propodium alone and to advance several centimeters by muscular action. Evidently, therefore, this specialized portion of the foot has considerable power of suction, although adhesion by means of mucus is in all probability the principal mode of attachment for the foot as a whole. When the snail is moving the anterior margin of the propodium is in close contact with the substrate and exhibits continuous though slight muscular contractions. It was conceivable, therefore, that it played some important part in effecting the locomotion of the animal which had been overlooked. In order to test this possibility the anterior portion of a snail's foot was removed so that the locomotor function became restricted to the mid and hind regions. Less than three days after the operation the snail was moving about the aquarium so much like other individuals that it could not be distinguished from them without examining its foot. When moving rapidly, ten records of its speed were taken which showed an average locomotor rate of 5 mm. in 3 seconds. Five records obtained at other times when it was most active averaged 2.6 seconds for the same distance. Another, normal animal, whose speed of locomotion was noted before the preceding tests were made, was moving at the rate of 5 mm. in 3.2 seconds (average of ten trials). After completing this experiment more of the anterior region of the foot was cut away so that there was nothing left which came in contact with the substrate which could possibly be regarded as a portion of the propodium. Again locomotion was not interfered with, and the snail glided both slowly and rapidly over the bottom of the aquarium. It is clear, therefore, that the propodium is not a part of the pedal mechanism which is essential for locomotion.

Muscular Locomotion.

In sharp contrast to the ciliary type of locomotion in *Polinices* is one involving the formation of rhythmic pedal waves. This is a muscular form of progression which begins by the posterior end of the foot being drawn forward initiating a dark transverse band or wave, which travels over the ventral surface of the foot until it reaches the anterior portion when the propodium is thrust ahead. At about the time the propodium is fully extended the hind end of the foot is again contracted and a second wave is produced. This muscular action causes a slight humping of the body, so that the shell rises after the posterior contraction and falls again as the wave passes forward. If the animal is moving very rapidly the anterior end of the foot may be lifted well above the substrate when the wave reaches it, extended and brought down again with considerable force. Although the wave may not be apparent along the lateral margins of the foot, it does at times extend to this region when, under favorable conditions for observation, it is perfectly clear that the wave is a band-like area of the foot which is temporarily raised above the substrate and moved forward. This interpretation of the pedal wave was offered by Parker in 1911, and later (1917) was shown by him to be true in the case of *Aplysia*. Olmsted ('17) also came to the same conclusion from his studies of Bermudian gastropods.

The speed of muscular locomotion was compared with that dependent on ciliary action alone. A snail whose foot measured about 10.5 cm. in length by 5.7 cm. in width was moving over the bottom of a lead-lined tank in which the water was over 3 cm. deep. By taking five records and averaging them, it was determined that five complete waves passed over the foot in 27.6 seconds, during which time the snail moved forward 7.8 cm. Thus a wave appeared on the average every 5.5 seconds and advanced the foot approximately 1.5 cm. It was moving, therefore, at the rate of one centimeter in about 3.5 seconds. A larger individual with a foot about 15.5 cm. long and 10.7 cm. wide moved much faster. When the results of five trials were averaged it was found that the snail was travelling at the rate of 18.5 cm. in 31.6 seconds, or stated as in the preceding case, a wave started every 6.3 seconds and moved the foot ahead about 3.7 cm. Accordingly the rate of locomotion was approximately a centimeter in 1.7 seconds. Comparing these locomotor rates with those determined for several snails moving without rhythmic contractions, it is evident that the muscular form of progression is faster. For example, the smaller, more slowly moving individual of the two noted above was timed as it glided by ciliary action over the glass bottom of a dish of sea water, a favorable substrate for this type of movement. Twenty records of its speed over distances of one centimeter were taken. The fastest noted was 5 seconds, whereas the series averaged 5.7 seconds.

Undoubtedly pedal cilia are beating when waves are passing over the foot, for they unquestionably are when the snail is burrowing into the sand and the same sort of contraction is in progress. The muscular form of locomotion may pass into the purely ciliary one by the gradual reduction of rhythmic pedal movements until they entirely disappear. Ciliary progression was observed more frequently than muscular in the case of snails confined in aquaria. The latter type now and then appeared when the animals were moving in a lead-lined tank, over a glass surface and often when they were climbing up the sides of glass aquaria. Ciliary locomotion was successfully accomplished over pebbly bottoms as well as sandy ones. In the former situation the foot, especially the propodium, molded its surface to the irregularities of the substrate and appeared as if it were flowing over them. The remarkable effectiveness of this form of progression is readily understood, however, if it is remembered that irrespective of the character of the substrate the cilia are always beating against a thick layer of mucus which adheres to the underlying materials, and forms a smooth bed over which the foot can readily glide.

If, when moving over a pebbly bottom, the propodium is thrust beneath the stones, the foot is worked forward by muscular contractions, the anterior end in contact with the glass bottom of the aquarium. Thus when considerable resistance is to be overcome the muscular form of progression is employed. This fact is well illustrated by the behavior of a snail burrowing into the sand. Rhythmic contractions go on as in ordinary locomotion. The propodium is directed downward, and when the pedal wave reaches the organ it is extended and worked farther forward and downward into the sand. By holding back a burrowing animal, sand grains may be seen travelling along the sole of the foot and also over the dorsal surface of the propodium, showing that the cilia are beating. By this procedure a snail may completely disappear beneath the sand in less than a minute and a half to a little over two minutes. When the anterior end of the foot was cut off, *Polinices* could not burrow successfully. By continued pedal contractions it was able to work into the sand somewhat, but at the end of about an hour it rested with a part of the shell still exposed. The propodium, therefore, although not necessary in locomotion, is essential for burrowing.

CILIARY CONTROL.

It is now well established that the transmission which excites activity over ciliated epithelia is not ordinarily directed through nerves, but through the protoplasm of the ciliated cells, a kind of transmission which on account of its resemblance to the nervous type has been designated neuroid. In the course of investigations on the behavior of cilia on the excised foot of the snail *Alectrion trivittata* (Say) the wave-like spread of action was clearly observed (Copeland, '19). Cilia quiescent over a limited area of the foot suddenly became active, others close by then began beating, and as the wave of action continued many were in motion. After a time they came to rest again. To account for this behavior, and the fact that cilia start beating over the entire ventral surface of the foot when the snail begins locomotion, one might assume that resting cilia are excited to movement in some definite region of the foot through impulses received from the nervous system, and that neuroid, or intraepithelial transmission, spreading from this center, brings about the activity of the remaining cilia. This possibility is rendered improbable, however, from the fact that if two observers are watching carefully the sand-covered surface of the foot of *Polinices* when the snail begins the righting reaction, no difference can be detected in the time when the sand grains begin movement, although the two points under observation may be ten centimeters apart.

In order to gain more definite information on the method of transmission in *Polinices* the following experiments were tried. After anesthetizing a snail in magnesium sulphate, the epithelium near the center of the foot was cut in such a way that an area 25 mm. long and 18 mm. wide was isolated from the surrounding epithelium. When the animal became active again after the operation the cilia on the isolated epithelium behaved the same as those over other parts of the foot, as far as could be determined by testing with sand grains. As long as the snail rested upside down there was no movement of the mucus or sand on the foot, but when it attempted to right itself ciliary activity began on the isolated patch of epithelium, as well as elsewhere, and continued until the animal rested again. Following this test more of the epithelium was cut away so that the subepithelial tissues were exposed in a well-defined band completely surrounding the epithelial island. The ciliary behavior was the same as before. The test was repeated on *Polinices heros,* an area of epithelium 13 to 15 mm. in diameter being separated from the surrounding portions near the center of the foot. Again the ciliary action over the isolated part was coördinated with that of the rest of the pedal surface. These experiments prove that impulses exciting ciliary action may be transmitted elsewhere than through epithelial cells, and point to a subepithelial or nervous pathway. Other tests on two examples of *Polinices heros* substantiated this view. The tissue lying beneath the epithelium was severed in a plane parallel with the surface. The cuts extended inward from near the border of the foot posterior to the propodium, leaving most of the overlying epithelium in contact with that of the rest of the foot, but isolating it from the deeper portions of the organ over an area, in one case, about 15 mm. long by 4 to 7 mm. wide, and in the second instance, approximately 8 mm. by 13 mm. Many tests were made during righting reactions, and although sand grains moved with the mucus directly in front and back of the areas cut under, and also to one side of them, they remained quiescent over the isolated regions themselves. The operation caused the epithelium to become somewhat contracted, but numerous tests on normal individuals have shown that a wrinkling of the pedal surface does not in any mechanical way inhibit the beating of cilia.

Conclusions.

The view that gastropod locomotion is not always a muscular process is well supported by the behavior of *Polinices* where two types of progression occur in a single species, one of which is immediately recognized as muscular, whereas the other presents entirely different features and upon analysis appears to be dependent on ciliary action.

The general ciliary behavior in *Polinices,* and especially the results of the experiments just described, uphold strongly the conclusions already drawn from a study of two species of the genus *Alectrion,* namely, that the control of ciliary action has been taken over by the nervous system, perhaps directly, or conceivably through connections with muscle or mucous cells associated with the ciliated cells. Intraepithelial transmission may play some part in effecting the spread of ciliary action, but evidently impulses directed over deeper pathways are the primary ones concerned in regulating this movement.

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THE EFFECT OF IODINE AND IODOTHYRIN ON THE LARVÆ OF SALAMANDERS. III. THE RÔLE OF THE IODINE IN THE SPECIFIC ACTION OF THE THYROID HORMONE AS TESTED IN THE METAMORPHOSIS OF THE AXOLOTL LARVÆ.*

E. UHLENHUTH.

(Received for publication September 11, 1921.)

The rôle of the iodine in the specific action of the thyroid hormone is still an undecided question. The most recent investigations on mammalia have led to the conclusion that the inorganic iodine as such is incapable of bringing about effects identical with the specific effects of the thyroid hormone.

Kendall[1] has shown that the specific reactivity of the thyroid hormone (thyroxin) as manifested by its capability of abolishing myxedema, mitigating cretinism, and raising metabolism depends chiefly on the presence, in it, of a CO-NH group. Iodine, although it may *increase* the specific reactivity of thyroxin, does not *produce* it, as is shown by the ineffectiveness of derivatives in which merely the hydrogen on the imino group has been replaced by acetyl without changing the position of the iodine atoms. Kendall, therefore, has been forced to conclude that the iodine is not responsible for the specific action of the thyroid hormone.

Quite in line with Kendall's viewpoint are Leo Loeb's recent experiments on the compensatory hypertrophy of the guinea pig's thyroid. Loeb found that the inhibitive effect in this kind of hypertrophy is specific for the thyroid hormone,[2] while inorganic iodine as such is incapable of preventing the compensatory hypertrophy of the thyroid gland. Leo Loeb, therefore, claims, like Kendall, that the effect of iodine is not identical with the effect of the thyroid hormone.

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* From the Laboratories of the Rockefeller Institute for Medical Research.

[1] Kendall, E. C., *Endocrinology,* 1917, i, 153–169, and 1919, iii, 156–163. Plummer, H. S., and Boothby, W. M., *Amer. J. Physiol.,* 1921, lv, 295.

[2] Loeb, L., *J. Med. Res.,* 1920, xli, 481–494, and 1920, xlii, 77–89.

As Leo Loeb rightly points out, the only facts seriously in the way of his own and Kendall's viewpoint are the experiments on amphibians, especially those of Swingle,[3] which have shown that administration of inorganic iodine causes precocious metamorphosis of tadpoles. Loeb overcomes this obstacle by assuming that the amphibian metamorphosis is not so much an indicator of thyroid activity as of the action of iodine as such. Kendall[4] likewise sees himself compelled to rule the amphibian metamorphosis out of the phenomena caused by the specific action of the thyroid hormone, and assumes now that thyroxin has a twofold effect, one on myxedema and basal metabolism, which is specific to the thyroid hormone and dependent on the peculiar chemical constitution of it, and one on the amphibian metamorphosis, which is merely due to the iodine contained in the thyroid hormone and not specific for the latter one, but for the inorganic iodine as such.

In a previous article I have shown that the interpretation given by myself and others to Swingle's experiments is not correct.[5] Although it may be true that the administration of inorganic iodine enforces metamorphosis of the tadpoles, it must be kept in mind that from the *effect of the administration of a substance* one can not, with any certainty, conclude upon the *effect of the administered substance,* unless the fate which this substance undergoes in the body is known. As to the fate of the inorganic iodine in the tadpole body, there is much evidence to show that it does not produce metamorphosis in the form of inorganic iodine, but after the thyroid—or in the absence of this gland other tissues similar in their function to the thyroid gland—has elaborated from it the thyroid hormone. The results on iodine-fed tadpoles, therefore, are neither new nor are they surprising as far as the ability of the tadpole thyroid goes to produce more "active thyroid hormone," if more iodine is administered; it is long known that the mammalian thyroid possesses exactly the same ability as shown by Marine and Rogoff in quantitative experiments.[6] The only thing new in Swingle's experiments is the discovery that the thyroid apparatus of the tadpoles or certain parts of it do not possess the ability of storing the thyroid hormone after its elaboration. The tadpole experiments in themselves, however, do not decide anything as to the rôle of the inorganic iodine in the action of the thyroid hormone. When viewed in the light of the experiments on salamanders they support the conception that inorganic iodine as such does not play an essential rôle in the effects characteristic for the thyroid hormone, and that the amphibian metamorphosis can be effected *only* by the thyroid hormone.

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[3] Swingle, W. W., *J. Exper. Zoöl.,* 1918–19, xxvii, 397–415.

[4] Kendall, E. C., *Amer. J. Physiol.,* 1919, xlix, 136–137.

[5] Uhlenhuth, E., Endocrinology (in press).

[6] Marine, D., and Rogoff, J. M., *J. Pharm. and Exper. Ther.,* 1916, ix, 1–10.

I have shown[5] that inorganic iodine, when administered to such amphibians in which the control of the excretion of the thyroid hormone is similar to that in mammalians—*i.e.*, in which no hormone can escape from the thyroid in the absence of the action of a particular releasing mechanism—is completely unable to produce the amphibian metamorphosis. In the experiments to be reported below I have used so-called axolotl larvæ. The axolotls, which are the larvæ of the species *Ambystoma tigrinum*, are characterized by an inhibition of metamorphosis resulting in a considerable prolongation of the larval period. It is possible, therefore, to extend, in this species, the experiments over long periods and to administer excessively large quantities of iodine. The ineffectiveness of such large quantities of inorganic iodine as compared to the prompt effect of amounts of iodothyrin containing only minute quantities of iodine disproves, in a quantitative manner, that inorganic iodine is the active principle of the thyroid hormone in the amphibian metamorphosis.

EXPERIMENTS.

From about 100 larvæ of the salamander *Ambystoma tigrinum*, which had developed into so-called "axolotl larvæ,"[7] 9 larvæ were selected for the experiment to be reported here. They were kept in ordinary tap water and fed earthworms in the beginning, beef liver later on.

Beginning of Experiment (December 9, 1920).

No. 1 measured 189.0 mm.; No. 2, 160.6 mm.; and No. 3, 194.4 mm. All three animals were completely larval (large gills, large

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[7] The axolotl larvæ were collected in the Colorado Rocky Mountains in August and September, 1920.

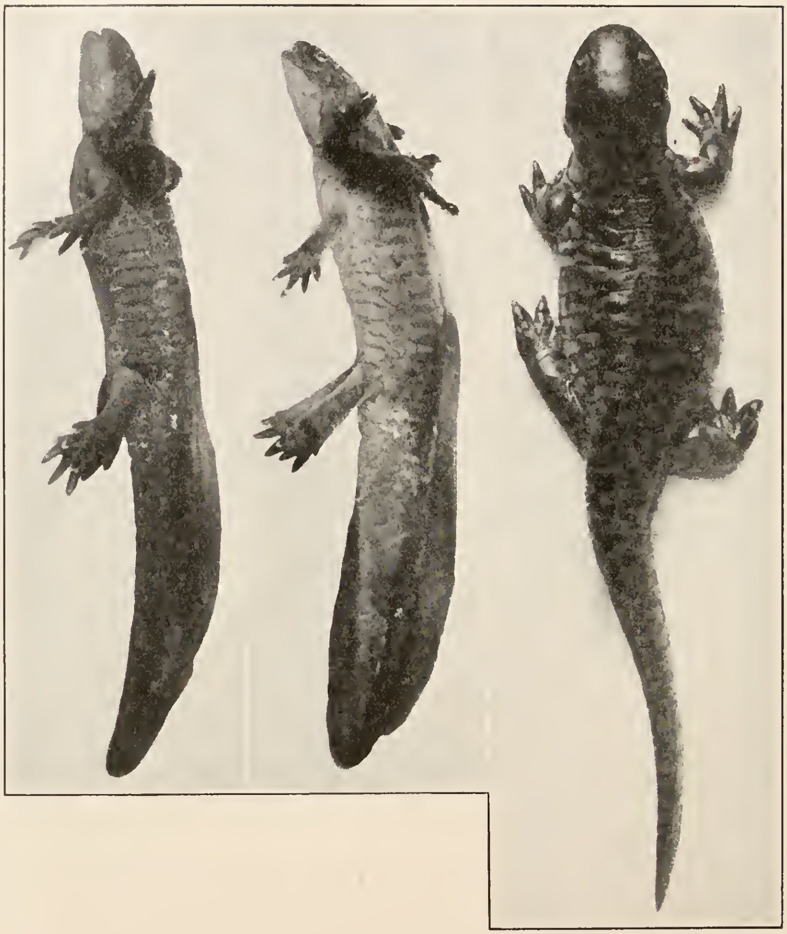

FIG. 1. Control larva 134 days after the beginning of the experiment. Note the shortened gills and the almost complete absence of the anterior portion of the ventral fin.

FIG. 2. Iodine-fed larva 134 days after the beginning of the experiment. Note the long gills and the completeness of the fin. This larva, although large amounts of inorganic iodine were administered to it, is less far advanced towards metamorphosis than the control animal.

FIG. 3. An animal to which iodothyrin was administered, photographed 134 days after the beginning of the experiment. This animal metamorphosed 13 days after the first administration of iodothyrin; it was a completely metamorphosed terrestric salamander when the photograph was made, and had been so several months before it was photographed.

fin on ventral and dorsal side of tail, eyes not bulging out) and No. 3 was neotenous (a sex mature male whose cloacal lips were swollen and warty). These 3 larvæ served as controls.

No. 4 measured 184.9 mm.; No. 5, 158.5 mm.; and No. 6, 214.0 mm. All 3 larvæ were completely larval; No. 6 was a sex mature male. This set of larvæ was used for the iodine experiment.

No. 7 measured 179.8 mm.; No. 8, 154.2 mm.; and No. 9, 184.5 mm. Again each animal was completely larval; the third one, however, presented no external sign of sex maturity (it was probably a female). This set was used for the iodothyrin experiment.

It will be noticed that each larva of each set was comparable to a corresponding larva of the two other sets, as regards size and stage of development. The larvæ selected for the iodothyrin experiment, however, were smaller in average than the larvæ of the two other sets. This selection was made in order to be sure that in case the larvæ kept in iodothyrin should metamorphose before the other larvæ their earlier metamorphosis was not due to a more advanced stage at the beginning of the experiment, but to the specific effect of the iodothyrin.

On December 9, 1920, the larvæ of the second set (Nos. 4, 5, 6) were placed into an iodine solution containing 5 drops of a 1/20 M stock solution of inorganic iodine (in 95 per cent. alcohol) per 1,000 c.c. of water, while the larvæ of the third set (Nos. 7, 8, 9) were put into water containing 0.1 gm. of Bayer's iodothyrin per 1,000 c.c. of water. The larvæ of the first set (Nos. 1, 2, 3) served as controls.

Thirteen Days after the Beginning of the Experiment.—The controls and the larvæ of the iodine experiment do not show any changes, in particular no signs of metamorphosis. Every one of the three larvæ kept in iodothyrin was found this morning to have shed its skin for the first time.

These three larvæ absorbed the gills very quickly, moulted several more times in rapid succession, and 25 days after beginning of the experiment had reached the stage at which the larvæ must be removed from the water and put on land, in order to prevent drowning. Fig. 3 shows No. 9 completely metamorphosed 134 days after the beginning of the experiment.

Forty-three Days after the Beginning of the Experiment.—Since 5 drops of iodine per 1,000 c.c. of water had no effect on metamorphosis, the iodine concentration was gradually increased and has reached today 9 drops in No. 5 and 8 drops per 1,000 c.c. of water in Nos. 4 and 6. Since it is believed that the alcohol used as solvent of the iodine might have some influence, too, on the animals, an equal number of drops of 95 per cent. alcohol is added from now on to the water in the controls.

Fifty-five Days After the Beginning of the Experiment.—The larvæ of the iodine series did not show any sign of an approach towards metamorphosis. Therefore the concentration of the iodine (and of the alcohol in the control series) was further increased and reached today 13 drops per 1,000 c.c. of water, a concentration of iodine more than 4 times the concentration sufficient to cause growth of the limbs in tadpoles.

Seventy-five Days After the Beginning of the Experiment.—The strength of the iodine solution was kept till today at 13 drops of a 1/20 M solution of iodine per 1,000 c.c. of water; in addition one larva (No. 5) was fed iodine crystals directly per mouth (1 crystal on the 57th, 63d, 68th, 70th and 74th day after the beginning of the experiment). Yet, in spite of these large amounts of inorganic iodine being adminstered to the larvæ, none of them shows any signs of metamorphosis so far.

From now on all larvæ, controls as well as experimentals, are kept in ordinary tap water, but the larvæ, Nos. 5 and 6 of the iodine series (No. 4 is used for a different experiment) are fed crystals directly per mouth.

One hundred and thirty-four Days After the Beginning of the Experiment.—The total number of iodine crystals fed to each one of the iodine-fed larvæ is now 25. This number of crystals was fed to No. 5 during a period of 76 days and to No. 6 during a period of 59 days. These large amounts of inorganic iodine were incapable of accelerating metamorphosis. In both the controls and the experimentals one larva has shed its skin, the iodine larva (No. 6) being now farther advanced than the corresponding control. But No. 5, the other iodine-fed larva, is completely larval and distinctly less far developed than the least advanced control larva No. 2.

One larva of each set was photographed today. Fig. 1 shows the least advanced control larva No. 2, Fig. 2 the least advanced iodine-fed larva No. 5, and Fig. 3 the iodothyrine-fed animal No. 9. While the latter animal is a completely metamorphosed terrestric salamander and has been so for several months, both the control and the iodine-fed animal are larvæ. The control possesses, however, slightly shorter gills, its eyes are slightly more bulging than those of the iodine-fed larva (not visible in the photograph) and the ventral portion of the fin is greatly absorbed as compared to the completely preserved fin of the iodine-fed animal. On dissection the small intestine of the control, although the control larva was larger than the iodine-fed larva, was found to be shorter (308.5 mm.) than that of the iodine--fed larva (387 mm.).

In short 134 days after the beginning of the experiment the least advanced control larva proved to be nearer metamorphosis than the least advanced iodine-fed larva, although the latter was kept, till the 75th day after the beginning of the experiment, in iodine solutions of concentrations up to 13 drops of 1/20 M solution of inorganic iodine per 1,000 c.c. of water, and in addition had received a total number of 25 iodine crystals during the last 76 days of the experiment.

Discussion.

The experiments reported in this article fully confirm the experiments reported in a previous paper, in which I showed that inorganic iodine does not enforce metamorphosis of normal larvæ of the salamander *Ambystoma punctatum*.[5] The administration of inorganic iodine, even if this substance be administered in comparatively large doses, is incapable of bringing about the amphibian metamorphosis in those species in which the control of the excretion of the thyroid hormone is of such a kind that no hormone can escape from the thyroid before the end of the larval period.[5, 8]

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[8] Uhlenhuth, E., *J. Gen. Physiol.*, 1919, i, 473–482, and *Am. Nat.*, 1921, lv, 193–221.

In the mind of the readers not quite familiar with the amphibian technique doubt may arise as to the correctness of the interpretation of the metamorphosis of one of the iodine-fed larvæ as not being due to the action of the iodine. I may mention, therefore that all of the axolotl larvæ, when kept under the conditions employed in the present control experiments, do undergo metamorphosis spontaneously. Consequently metamorphosis of the iodine-fed axlotl larvæ could be interpreted as the result of the iodine administration only, if it would have occurred at a greatly precocious date and simultaneously or nearly simultaneously in all the experimental larvæ. Not only were these conditions not fulfilled, but one of the experimental larvæ did not metamorphose at all and, at the termination of the experiment was farther away from the metamorphosis than any of the control larvæ, although observation extended over a period of more than 4 months, during which time large amounts of iodine were administered.

That the dosis of iodine was too small to enforce metamorphosis, can not be assumed; for, as will be discussed presently, the dosis of iodine administered to the larvæ of the iodine series was far in excess over the quantity of iodine contained in the dosis of iodothyrin which caused a very rapid metamorphosis.

Furthermore it is impossible to explain the ineffectiveness of the inorganic iodine by assuming that the larvæ employed in the iodine experiment had entered a stage at which the responsiveness to the stimuli causing metamorphosis has been lost. Not only could metamorphosis easily be produced by iodothyrin, but occurred spontaneously in the controls and in many other axolotl larvæ comparable to the iodine-fed larvæ in every respect (except for the iodine administration).

Hence it is evident that inorganic iodine is incapable of causing metamorphosis of the salamander larvæ.

Regarding the rôle of the iodine in the thyroid hormone these experiments also show, in a very striking manner, that the effect of the iodothyrin is not due to its iodine content. If it were due to its iodine content, quantities of inorganic iodine equal to those contained in an effective dosis of iodothyrin should cause metamorphosis. This expectation, however, is not fulfilled in the results of the present experiments. Bayer's iodothyrin, according to Bayer's "Materia Medica" and according to Baumann's own description of this substance,[9] contains 0.3 mgm. iodine per 1 gm. of substance. Consequently a dosis of 0.1 gm. of iodothyrin, which causes very rapid metamorphosis of the axolotl larvæ, contains 0.03 mgm. iodine per 1,000 c.c. of water. The weakest solution of inorganic iodine employed in the iodine experiment (5 drops of a M/20 iodine solution per 1,000 c.c. water) contained approximately 1.0 mgm. of iodine per 1,000 c.c. of water (1 drop being equal to about 0.2 mgm. of iodine), while the strongest solution contained even 2.6 mgm. iodine per 1,000 c.c. water. In addition to these quantities of inorganic iodine the larvæ of the iodine series received, directly per mouth, a total amount of 25 iodine crystals which equals approximately 40 mgm. of iodine, the average weight of one crystal being 1.6 mgm. Yet these quantities of inorganic iodine did not cause metamorphosis, although they were greatly in excess over the quantity of iodine contained in an effective dosis of iodothyrin. It is obvious that it is not the quantity of iodine contained in the iodothyrin, which produces the effectiveness of the latter substance.

Lenhart's experiments[10] showed that the effectiveness of thyroid gland in the amphibian metamorphosis of tadpoles increases with an increasing amount of iodine. These experiments are frequently quoted to demonstrate quantitatively that the active substance of the thyroid hormone in the amphibian metamorphosis is iodine. In the light of the present experiments this interpretation of Lenhart's experiments seems untenable. As pointed out in the introduction of this article, it is probable that the inorganic iodine does not produce the metamorphosis of tadpoles in its inorganic form, but after the thyroid apparatus has elaborated from it the thyroid hormone. Therefore Lenhart's experiments may be simply the expression of the fact that the tadpole thyroid, as claimed by Swingle[11] elaborates more hormone if more iodine is available. Moreover it is very probable that in Lenhart's experiments an increased amount of iodine contained in the specimens of thyroid gland which were fed to the tadpoles corresponded to an increased amount of thyroid hormone (A-iodine, Kendall); in this case Lenhart's experiments would mean that metamorphosis is the more accelerated, the more thyroid hormone there is supplied to the tadpole, a result which corresponds with quantitative experiments on the action of iodothyrin in salamanders[5] and does not throw any light on the rôle of iodine in the thyroid hormone.

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[9] Baumann, E., and Roos, E., *Zeitschr. f. physiol. Chem.*, 1895–96, 481–493.

[10] Lenhart, C. H., *J. Exper. Med.*, 1915, xxii, 739–746.

[11] Swingle, W. W., *J. Exper. Zoöl.*, 1919, xxvii, 417–425.

Swingle[12] in support of his viewpoint that inorganic iodine as such causes the amphibian metamorphosis, has emphasized the specificity of iodine, the administration of inorganic bromine being incapable of causing metamorphosis. It is not impossible, however, that this fact merely means that the organism does not possess a mechanism by means of which bromine can be employed in the manufacture of thyroid hormone; if it were possible to make synthetically, outside the organism, a substance identical with Kendall's thyroxin in every respect, but possessing a bromine atom in place of every iodine atom, this substance may be capable of producing metamorphosis.

SUMMARY.

1. Iodothyrin and inorganic iodine, in known quantities, were administered to so-called axolotl larvæ of the salamander *Ambystoma tigrinum.*

2. A dosis of iodothyrin containing only 0.03 mgm. iodine per 1,000 c.c. of water caused metamorphosis 13 days after its first administration.

3. A dosis of inorganic iodine 33 to 86 times larger and feeding still larger doses directly per mouth did not cause metamorphosis.

4. The amphibian metamorphosis is truly the expression of the thyroid activity and not the result of the effect of inorganic iodine.

5. Inorganic iodine as such is not the active principle of the thyroid hormone.

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[12] Swingle, W. W., *J. Gen. Physiol.*, 1919, i, 593–606.

Vol. XLII *April, 1922* *No. 4*

BIOLOGICAL BULLETIN

A WEEK WITH A MINING EUMENID: AN ECOLOGICO BEHAVIOR STUDY OF THE NESTING HABITS OF ODYNERUS DORSALIS FAB.

C. H. TURNER,

SUMNER TEACHER'S COLLEGE, ST. LOUIS, MO.

HABITAT.

At the edge of an extensive bottom-land farm near Lebanon, Ill., there is a large barren tract of land (Fig. 1, *B*). Its northern portion, which resembles roughly the continent of North America, is 80 feet long, 42 feet wide at its greatest breadth, and 9 feet at its narrowest. From the end that is 9 feet wide an arm 300 feet long tapers gradually until it is only 4 feet wide. In August, 1921, to the west and to the south of this desert there was a bottom-land cornfield covering several acres. The ascending slope and the upland on the east were covered with a dense growth of field sorrel, variegated with scattered iron weeds and mallow plants. On the western half of the northern boundary there was a cornfield, and on the eastern portion of that boundary there were plants similar to those on the east. Near the eastern edge of the barren tract the weeds were dwarfs, but they rapidly increased to normal size. From northeast to southwest and then south a small stream meanders through this land. The northern, especially the northeastern portion, is slightly higher and decidedly drier than the remainder. In meandering through the northeastern portion the streamlet maintains a uniform width of about six inches; in the remainder of the area it frequently widens into shallow lakelets. The flow of the water is continuous, but gentle. This apparently insignificant stream, which is the outlet of a spring with a decidedly salty taste, is the cause of all this barrenness. The minerals it conveys from that spring have destroyed the vegetation.

On the nearly level, but slightly higher, land about the headwaters of this brooklet, on the 26th of August, 1921, three colonies of *Odynerus dorsalis* were discovered (Fig. 1, *B, a, c, c*). One of these (Fig. 1, *A*) was on a spot that was absolutely barren; the other two were in spots the barrenness of which was partly interrupted by low-growing weeds (Fig. 1, *C*). At no other place in that desert strip, nor on any of the land for a half mile around, were there any colonies of this species. There, and at no other place in the neighborhood, were found the three essentials for the existence of this species: a patch of hard, dry, land, a near-by body of water, and a field infested with Hesperid caterpillars.

Evidently such a locality—a barren, or nearly barren, patch of dry, hard, ground, near-by water, accessible Hesperid larvæ—is the normal habitat of this species, for it is in just such situations that it has been found by Isely ('14, p. 282) and by the Raus ('18, p. 312). Hartman's statement ('05) that the species constructs mud nests on grass stems is the only discordant note in the literature, but his experience is radically so different from that of Isely, the Raus, and me that it is hard to believe that he is discussing the same species. Indeed, in a letter to Mr. Rau, Hartman states that it is probable that the species he discusses had been wrongly classified.

If one thinks in terms of social bees and hornets, the word populous could hardly be applied to these colonies; but they were slightly larger than those seen by Isely. Isely ('14, p. 293) writes: "*O. dorsalis* has a tendency to nest in colonies. I sometimes found an isolated nest, but usually the nests were in small groups, sometimes as many as eight. Usually only two or three wasps would share an open space in a pasture, each wasp digging one or two nests. I never found them in populous colonies like those of *O. papagorum*." In speaking of *O. papagorum,* he says (*op. cit.,* p. 256): "Hundreds of them were at work there in the high banks of light earth." In the three colonies observed by me the number of wasps varied from twelve to sixteen. This estimate was made not by counting the wasps, but by noting the number of holes that were in process of construction or of being provisioned at the same time.

Description of the Nests.

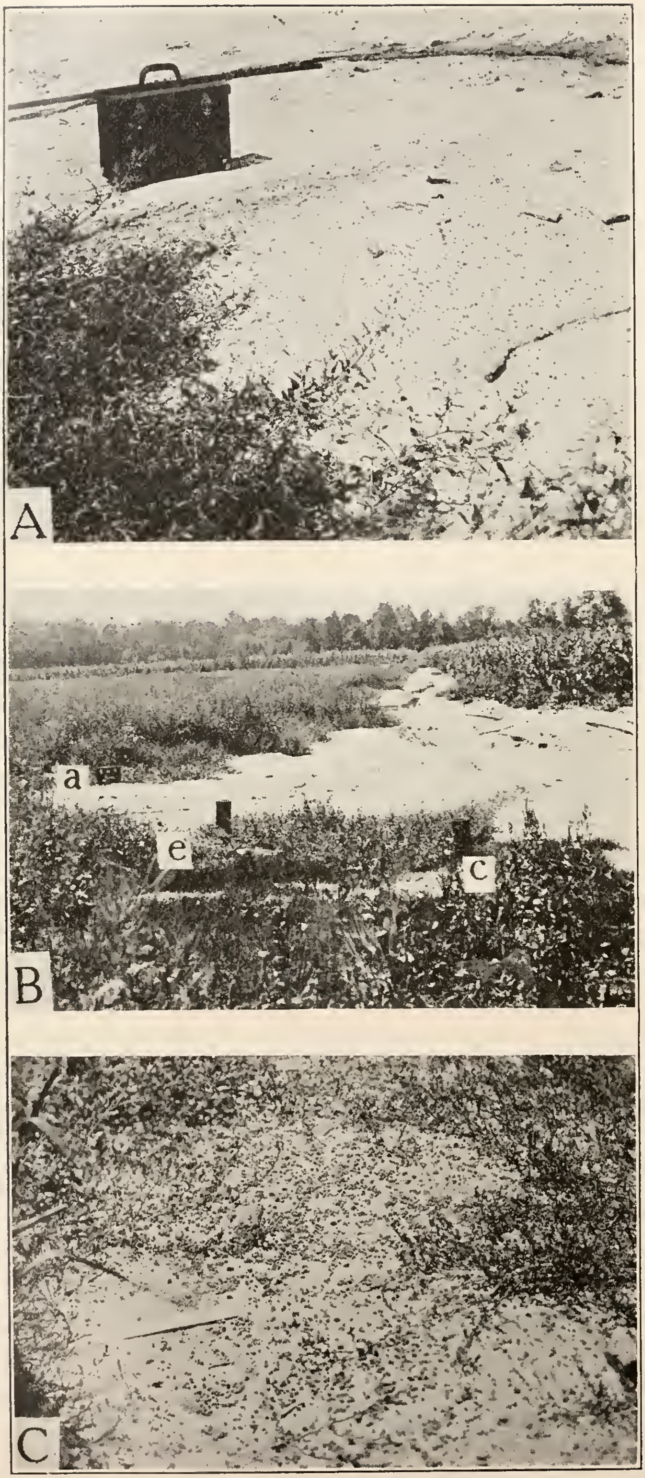
Each nest consists of one to three, usually two, barrel-shaped or sub-spherical cells for larvæ and a small air chamber, the latter being above the former. Thin partitions of clay separate the compartments from each other and a clay plug separates the whole from the outer world (Fig. 6, *B–D*). In the cases observed by me, when there were three cells, the upper was empty; when there were two cells, the upper was smaller than the lower. These nests vary in length from one and a half to two and three fourths inches. Each cell is about five eighths of an inch wide and the entrance to the nest is about three eighths of an inch. The transverse partitions are thin. Each wasp constructs a succession of nests which are arranged close together. These facts were ascertained by excavating twenty-five nests.

The above description harmonizes perfectly with Rau's observations and with the majority of Isely's; however, the latter author found some nests with as many as seven cells.

Excavating the Nest.

The nest-building behavior of these wasps is fascinating. Preliminary to digging, the wasp makes sweeping movements over about forty square inches of surface. She moves about here and there as though testing the ground. The purpose of this survey I do not know; it may be that it enables her to detect the spots where other nests are located. This surmise is supported by the fact that excavations demonstrate that the nests occur in small groups. The nests of each group are separated from one another by walls that are not very thick; yet I have never discovered one burrow running into another.

The preliminary explorations over, the wasp makes a flight of orientation and then flies to the near-by streamlet and takes a deep drink of water. Returning to the spot selected, she moistens the ground and, with her mandibles, removes several pellets of dirt and conveys them, one by one, to a distance of from two inches to two feet. She then goes for some more water and then returns to the excavating (Fig. 3, *a*). This is repeated over and over again until the lower cell has been completed and the next one partly finished.


A
a
e
c
B
C

It usually takes a little over an hour to reach this point in the digging of the burrow. In removing the dirt she goes in head first and comes out backwards.

Isely thinks that this species carries the water in her jaws. He says ('14, p. 286): "Mrs. Wasp flew in the direction of the river and returned with her mouth parts glistening." Although I watched the wasps carefully, I failed to detect water glistening on their mouth parts. However, I know that they carry water to moisten the clay; because, immediately after a wasp that has returned from the streamlet applies her head to the ground, the clay glistens for a moment and then becomes dull. The water sparkles until it is absorbed by the clay. Hence I am inclined to believe, with the Raus ('18, p. 314), that the water is carried in some part of the digestive tract (probably the crop).

In a previous paragraph I have stated that each pellet, as it is removed, is carried to a distance of from two to twenty-four inches and deposited. How carried? According to Isely ('14, p. 285), she flies and drops them all, in about the same place, at approximately eighteen inches from the nest. The Raus ('18, p. 314) write: "She would then back out of her hole with a round, well-formed pellet of mud in her mandible, always fly from two to fifteen inches and drop it." They do not state whether the pellets are scattered around or deposited in one place, but the illustration furnished pictures them distributed, in a single layer, over the ground. Of the colonies I am describing, in one (Fig. 1, *B, a*) the pellets were scattered, in a single layer, over the plot; in the other two, in addition to the scattered pellets, there were piles of them near certain nests. Some wasps were flying and dropping the pellets, some were walking from the nest and depositing the pellets and then flying back to the nest, some were walking from the nest, depositing the pellets, and then walking back to it. It

FIG. 1. Views of the habitat of the colonies studied. *A*. Near view of the colony located at *a* in *B*. The specks on the ground are pellets dropped by the wasps. *B*. View of the environment of the three colonies: *a*, location of a colony situated where the ground is absolutely free from vegetation; *c*, location of a colony where a few low-growing weeds partly cover the ground; *e*, a colony similar to *c*. *C*. A near view of the colony located at *c* in *B*. The specks on the ground are pellets dropped by the wasps.

was readily seen that the piles of pellets were formed by the wasps that were walking and depositing pellets. Careful observation demonstrated that the piles of pellets were always associated with those nests that were partly covered by the low weeds growing on certain portions of plots *c* and *e*. What is the cause of this striking difference in the behavior of these wasps? Are some members of the species born with an instinct for flying and scattering pellets and other members of the same species born with an instinct for walking and depositing pellets, or is there some relation between the partial covering of nests by low-growing weeds and the change from flying to walking?

EXPERIMENTAL INVESTIGATION OF THE PLASTICITY OF THE CARRYING BEHAVIOR.

If some members of this species are born with an inflexible instinctive tendency to fly and scatter pellets and other members of the same species are born with a fixed instinct to walk and deposit them in piles, nothing in the way of an experiment can alter matters; but if, in response to a certain environment, the walking form of behavior has been derived from the flying, then it should be possible to experimentally demonstrate it. Since the low-growing weeds do not make flight impossible, if they are the cause of the change in behavior it must be because they render flying with a load so unpleasant that the insect takes to walking to avoid the unpleasantness. The problem that faced me was to devise some apparatus that would permit the insect to fly, but which, at the same time, would render flying with a burden so unpleasant as to cause the wasp to abandon the attempt.

In the experiments I used what might be called an interference maze, constructed out of stove wire. The maze was constructed in the field. A burrow was selected from which the wasp was flying and scattering pellets. Several pieces of wire were stuck, near together, in the ground near one of the nest openings. These wires were then so bent as to radiate over the burrow like the rays of a fan. Near the nest these wires were only an inch, or a little less, above the surface of the ground. As they extended outward they became gradually higher and higher until they reached the point where they were bent abruptly to form a support. A series of sub-parallel, semi-elliptical wires were constructed across this fan-like expanse of wires (Fig. 2).

A number of these interference mazes were used. They were all similar, but no two were identical (Figs. 2 and 3 are from the same maze and Figs. 4 and 5 are from another). The only points invariably adhered to were two: first, in the immediate vicinity of the nest the wires were so arranged as to make it difficult for the wasp to get a flying start with her pellet of mud; second, the meshes were made sufficiently open for the wasp to fly through. Two mazes were used at a time. Each experiment extended over a period of from one to two hours. Three days were devoted to these experiments. The wasps, which invariably spent the night in the nests, became active between 7:30 and 8:00 A.M. and continued to work until about sundown. Each day the experiments were begun as soon as the wasps became active. On the first day they were continued until they became inactive; on the two other days I stopped work between four and five in the afternoon. Since two mazes were used at a time, this gave time enough for fifteen experiments.

The results of these experiments were conclusive and positive and sufficiently uniform to be epitomized in a single paragraph. In each case the wasp flew away with a few pellets (usually less than six) and then began to walk. In the midst of this uniformity there was individuality. The majority of the wasps walked from the nest, deposited the pellet (Fig. 2, *f*), and then walked back to the nest; some walked from the nest, deposited the pellet, then flew out of the maze and back to the nest. One walked to and from the pellet pile most of the time, but occasionally it would fly and scatter a pellet. Another would walk from nest to pellet pile and back until it came time to get some more water, then she would fly with the pellet out of the maze, drop it, and then go for water. Thus we have conclusive experimental proof that the walk-and-deposit-the-pellet behavior of these wasps has been derived from the fly-and-scatter-the-pellet behavior in response to an environment which made flying with a load unpleasant.

Occasionally, in arranging the maze over a certain nest, the

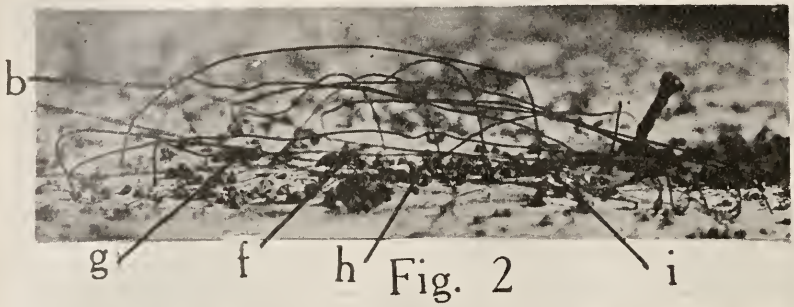


Fig. 2

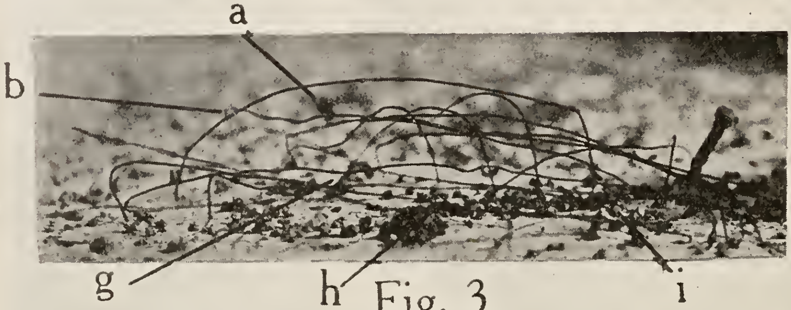


Fig. 3

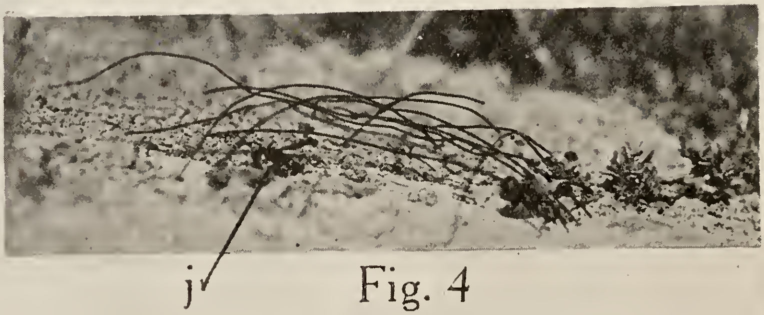


Fig. 4

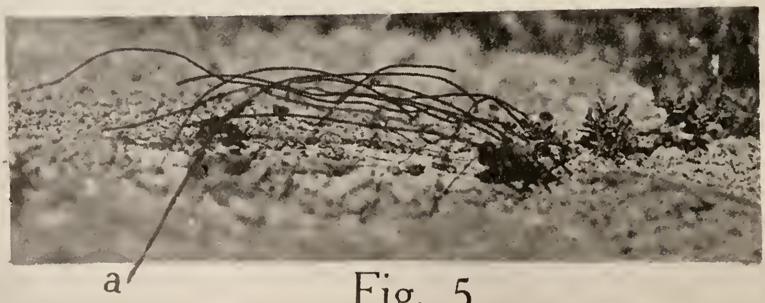


Fig. 5

higher portion of the maze would cover another nest in which the wasp was still digging. In such cases the second wasp would continue to fly and scatter pellets (Fig. 4, *j*; 5, *a*). Thus, in the same maze, I sometimes had one wasp walking and depositing pellets in a pile or piles and another flying and scattering them.

It is not claimed that these experiments predicate of wasps the power of logical thinking; on that topic experiments of this type are non-committal. However, they demonstrate conclusively that, in *Odynerus dorsalis* Fab., the walking-to-deposit-the-pellet behavior is derived from the flying-to-scatter-the-pellets activity, in response to a slight change in the environment.

Experimental Interference with the Wasp When Excavating.

A series of experiments was conducted to test the reactions of excavating wasps to objects dropped into their burrows. Three kinds of objects were used in these experiments: pellets of dirt (mineral matter), short pieces of weed stems (plant matter), live caterpillars (animal matter).

Experiments with Pellets.—*Pellets of dirt that had been removed from nests by certain wasps were dropped into the partly excavated nests of other wasps.* In each case the wasp removed the pellet and disposed of it in the usual manner. In each case the return to the surface with the pellet was too quick to permit the formation of one.

Experiments with Bits of Weed Stems.—*Stems of the weed were cut into pieces about two inches long. These were placed in the partly completed burrows of the wasps. In some cases the*

Fig. 2. Showing a wasp that has been induced, by the maze, to walk and deposit her pellets: *b*, maze; *f*, wasp carrying the pellet; *g*, opening of the nest; *h*, piles of pellets deposited by the wasp; *i*, another wasp entering her nest to oviposit.

Fig. 3. Showing a wasp, that had been excavating inside the maze, flying for water: *a*, the wasp flying for water; *b*, the maze; *g*, entrance to the nest; *h*, piles of pellets deposited by the wasp; *i*, a wasp leaving her nest after ovipositing.

Fig. 4. Showing a wasp preparing to fly off with a pellet: *j*, getting ready for flight.

Fig. 5. Showing a wasp flying away with a pellet: *a*, a wasp flying.

nest was so shallow that only half of a wasp could be hidden in it; in others the burrow was just deep enough to include the whole of the wasp. Invariably the wasp removed the bits of weed and deposited them on the ground near by. Sometimes the wasp removed the weed stem or stems immediately; at others she continued excavating for a short time and then removed the foreign substance. It seems as though she removed the stems as soon as they greatly hampered her movements.

EXPERIMENTS WITH CATERPILLARS.—*Home-coming wasps were robbed of their prey until I had collected a dozen paralyzed caterpillars. Burrows in two stages of completion were selected: those that were just deep enough to half conceal the worker and those that were sufficiently deep to just hide the wasp. While the wasp was gone for water one of the caterpillars would be placed in the burrow and the nest watched until the wasp resumed her excavating. This was repeated over and over until the twelve caterpillars had been used.* With the exceptions of touches of individuality the behavior was invariably the same. The wasp always removed the caterpillar from the nest, flew with it to the weed-infested field, and returned empty-handed. In some cases the wasp flew away with the caterpillar as soon as arriving at the surface of the ground; more often she would climb some weed before flying away. Sometimes the journey was preceded by a longer or shorter flight of orientation, at others by none. The final flight was sometimes in one direction and sometimes in another, but always in the direction of the weed-infested field. Why did these wasps react so differently toward the several classes of objects? When pellets of earth were dropped into the nests, the wasps either flew and scattered them or walked and deposited them in piles; when short pieces of weed stems were placed in the burrows, the wasps deposited them on the ground adjacent to the nest; when paralyzed caterpillars were placed in the holes, the wasps flew with them to the insect-infested field, which was several yards away. I confess that every time a wasp flew away with a caterpillar it gave me an intellectual thrill; but, to me, the reason for the behavior remains a mystery.

Homing Experiments.

While observing these wasps excavate their nests I was deeply impressed by two things: the facility with which the wasp, on returning with water, located her nest in the midst of surroundings which, to my senses, were almost uniform, and how easily she could be confused by slight changes in the environment. Almost any disfigurement of the surface of the ground would cause her to have trouble in locating her nest. Small pieces of tile and nails used to mark certain nests and radiating lines which I scratched about others caused confusion. In each case the reaction was the same. Arriving in the vicinity of the nest, the wasp would circle about in an irregular manner, sometimes afoot and sometimes awing, as though lost. After a certain length of time it would either find the nest or else abandon the attempt and start another. If the nest were found, before taking another trip afield, the wasp would always make a careful flight of orientation.

In the following words Isely ('14, p. 294) states how easily the wasps he studied were affected by changes in the surface of the ground: "Although not generally sensitive to observation, *O. dorsalis* was more responsive to changes in the surroundings of her burrows than was *O. papagorum*. A few marks with a knife, to assist in locating a nest at a later time, seemed to disturb one wasp considerably. On her return to the nest she made a prolonged flight of irregular circles above her nest, while she was in the habit of alighting without any hesitation. On another occasion I mutilated slightly the entrance to a burrow. Upon the wasp's return she circled around the burrow a few times and then alighted about two inches from the entrance. She flew away and returned in about a minute and repeated the observation performance. Again she flew away and returned without entering. This time she apparently deserted her nest."

Evidently, when in the neighborhood of the nest, *Odynerus dorsalis* finds her way by means of landmarks. How keen, then, must be her powers of observation since she sees landmarks in a situation where we see only uniformity!

Provisioning the Nest.

The nests are stored with a black-headed, green, Hesperid larva, which the wasp obtains from the neighboring field. These are paralyzed and packed in the cell. The wasp carries the caterpillar dorsal side down and head to the front. When she reaches the burrow she pushes the caterpillar in ahead of her. Sometimes at one stage and sometimes at another in the provisioning of the nest the wasp folds her wings close to the body and backs into the nest (Fig. 2, *i*), attaches her egg to the roof of the cell by means of a cord about as long as the egg, and then comes out head first (Fig. 3, *i*) and resumes her work. This statement is an assumption based on the following facts: There is never found more than one egg suspended from the top of a cell; only once during the provisioning of the cell does the wasp back in; the attitude attained by backing into the cell is the most favorable one for attaching the egg to the roof. When this lower cell has been filled, the wasp fetches some water and then, out of the scrapings from the walls of what is to be the next cell, she covers the first with a clay wall. In most cases, after this partition has been formed, the second cell is the right size for receiving the provisions; occasionally the wasp must remove a few pellets before storing it with caterpillars. After it has been provisioned a mud cover is made for it out of scrapings from the wall of the upper portion of the burrow. The burrow is then closed with a plug of mud and dirt. The material to form this plug is scraped from the rim of the burrow; as a result the freshly formed burrow is topped with a saucer-like depression. After a few days, or even a few hours, the wind, the rain, and the pellet-dropping activities of other wasps obliterate the depression. To obtain sufficient water for the softening of the clay the wasp makes frequent trips to the near-by streamlet. During the whole time that the provisioning is progressing the burrow remains wide open. The above account tallies with those of Isely ('14, pp. 289–290) and the Raus ('18, p. 320).

Experimental Interference with the Provisioning Activities of Wasps.

While the wasp was in the nest arranging her provisions from one to four caterpillars were dropped into the burrow. Some of *these larvæ were obtained by robbing home-coming wasps; the majority were obtained by excavating nests that had been stored and sealed. Fifteen such experiments were performed.* On one occasion, when I had dropped two caterpillars into a nest, the wasp left the burrow and went hunting without removing them. Later in the day she took two caterpillars from her nest and carried them to the field; but there is no way of telling if these were the two added. On another occasion, when three caterpillars had been dropped into a nest and two deposited on the ground near the nest, the wasp, on emerging, captured one of the caterpillars that had been placed on the ground near the nest, flew with it to a near-by weed, stung it, malaxated it, and then carried it into the burrow. Immediately she emerged with a caterpillar and flew with it to the near-by field. She then continued to remove caterpillars from the nest until she had conveyed five to the field. In all other cases the wasp carried the caterpillars afield as soon as they had been dropped into the nest. It seems that the wasp is averse to using caterpillars that have been captured by some other wasp, and that she has some means of distinguishing them.

To see if she could recognize her own captures, I frequently removed the prey from a home-coming wasp and dropped it on the ground among the nests. Invariably she would search until she found the caterpillar and then would carry it to her nest.

With a pair of forceps I occasionally attempted to remove the caterpillar from a wasp that was walking on the ground near her nest. She would make strenuous efforts to retain her hold, occasionally stinging the larva. If I succeeded in removing the caterpillar, the wasp would search for it until found and then store it in her nest.

Isely ('14, p. 294) states: "*O. dorsalis* never seems to have any difficulty in finding her way to her burrow after a field trip. Usually she flies directly to it; I never saw her do otherwise when she was returning from the field with prey. At times, when she returned from the field unladen, I have seen her pause at another burrow in the colony. This action may have been prompted by curiosity instead of being a mistake in location." In that same connection the Raus write ('18, p. 320) : "These wasps, when returning from the field, seem to experience no difficulty whatever in locating their burrows. They carry their prey on the wing, usually with comparative ease, hugging it tightly all the while, alight at the brink of the hole and push it in ahead of them, holding it as they lower it; then they follow it into the hole, remain inside a few seconds—perhaps a half minute—and then come backing out. Sometimes the wasp soars away directly in quest of other game; at other times she sits down and washes her face for a moment, then rises and poises on vibrating wings an inch or two above the hole, turns around on the wing as if inspecting the site, then circles about and flies away. She is calm, gentle, and composed in all the maneuvers, betrays no nervousness, and wastes no time in blustering." Since I had read both of these accounts before making my observations on the same species, I was surprised to find that wasps coming from the field did sometimes have difficulty in finding their nests. My wasps, when arriving laden from the field, sometimes went direct to the nest and pushed the caterpillar in; but usually they did not. Frequently a wasp would examine the entrances to several nests before entering one. At other times a wasp afoot and awing would circle around and around for several minutes before entering the nest.

Movements as conspicuous as these, if performed by the wasps they studied, could not have been overlooked by such keen observers as Isely and the Raus. Why should my wasps behave so differently in this respect? I think I have the solution. In arranging mazes for experimental work, and in marking certain nests with bits of tile and with nails, I was continually disfiguring the ground. Since my wasps spent from ten minutes to more than half an hour on each hunting trip, it is quite likely that during the absence of a wasp the ground would be changed sufficiently to confuse her. A careful consideration of the records of my first afternoon's work confirms this opinion. Then the ground had not been disfigured with pieces of apparatus. To see if the colony was worth careful study, I made a few observations and marked a few nests. In most of the records of that afternoon the caterpillar-bearing wasps are described as going direct to their nests. Two records tell a different story and those two were connected with nests that had been marked. I shall transcribe both of those records, because they have a direct bearing on this point. We shall call them records of wasp *A* and *B*.

Record of wasp A:

11:20 A.M. The wasp places a caterpillar in the nest and departs for the field. *I place a small piece of tile near the opening.*

11:30 A.M. The wasp returns with a caterpillar. She does not recognize the nest. She spends thirty minutes returning over and over to the same spot, but fails to recognize the nest.

12:12 P.M. *I remove the piece of tile.* Within two minutes the caterpillar is carried into the nest.

Record of wasp B:

11:34 A.M. The wasp carries the caterpillar into the nest, makes a flight of orientation, and then flies afield. *I place a small piece of coal to mark the nest.*

11:44. The wasp returns with another caterpillar, goes to the wrong hole, and, still bearing the caterpillar, flies about in irregular curves.

11:45. The wasp starts into the hole, leaves it and tries another. Still bearing the caterpillar, she flies about.

11:58. The wasp deposits the caterpillar in the nest and goes for water.

11:59. The wasp returns and begins to scrape the rim of the burrow and to add the material to the inside of the nest.

Evidently the wasp when returning with a caterpillar is just as sensitive to changes in her environment as she is when excavating.

I have a very strong suspicion, but not positive proof, that these wasps sometimes carry the caterpillar into the wrong nest. Occasionally I have seen a wasp, belonging to some nest upon which I had not experimented, remove a caterpillar and fly with it to the field. The Raus ('18, p. 324) have noticed the same thing. They write ('18, p. 324): "From one hole we saw an *O. dorsalis* emerging and watched her closely to ascertain her method of egress. Imagine our surprise when we saw her carry out a *P. catullus* caterpillar in her front and middle pair of legs, pause at the brink of the hole for several seconds to adjust the caterpillar properly between her forelegs and mandibles, and fly away with it." The behavior of these wasps is identical with that of those into whose nests I had introduced caterpillars that had been captured by other wasps. It seems reasonable to assume that these caterpillars have been deposited in the nest by some other wasp.

Time Required to Provision the Nest.

Isely found ('14) that many of the specimens examined by him excavated, provisioned, and sealed the burrows in less than two hours, although some of the individuals took a day for the work. In every case that I followed from beginning to the end the wasp always constructed the burrow one day and sealed it on the afternoon of the next day or the morning of the day after. Thus the time elapsing from the beginning of the excavation to the final sealing of the nest was twenty-four hours or more. Since the amount of time consumed in excavating the burrow was about the same in each case, the greater length of time required by my wasps to complete the work may have been due to lack of food. Isely's colonies were located where the mallow was abundant; my wasps were situated near a large field of field sorrel, scattered through which were a few mallow plants. On one occasion I followed a wasp, from plant to plant, for more than half an hour. She examined leaf after leaf, without finding any caterpillars. I uncoiled several leaves, but found them empty.

Capturing the Prey.

Isely ('14, p. 289) writes as follows: "When *O. dorsalis* would come upon a crumpled leaf containing the larva of the spotted skipper, she would commence tearing energetically at the silken nest, first at one end and then at the other. Although the wasp worked furiously and without pausing, sometimes more than five minutes were required to dislodge the caterpillar. Usually, however, in less than a minute the caterpillar would be jerked violently from the cover, seized by the neck, and stung two or three times under the thorax. Once I saw a wasp seize a caterpillar by the tip of the abdomen, to jerk it out of the nest, and sting it under the last abdominal segments. Then she quickly seized the neck and gave it three thrusts under the thorax. A vigorous malaxation invariably followed the stinging. The capture of a caterpillar usually caused considerable excitement on the part of the wasp. Sometimes she would lose her footing and both insects would roll from the leaf to the ground before the victim could be subdued."

Although I have watched this wasp searching the crumpled leaves for caterpillars, I have never been on hand at the moment she captured her prey. However, I have witnessed both the stinging and the malaxation of the victim. The first time I noticed the malaxation the wasp and the caterpillar were on the leaf of a weed and the wasp was vigorously crushing the neck of the larva with her jaws. Then the body of the caterpillar was moved forward until every portion of the body had been crushed by the rapidly moving mandibles. I witnessed the stinging on two occasions. Once a wasp captured a paralyzed caterpillar which I had placed on the ground near her nest. The larva was seized by the neck, the abdomen of the wasp curved beneath the caterpillar, and the sting of the wasp thrust into the thorax three times. On another occasion, by means of forceps, I was attempting to pull a larva away from a wasp. Suddenly she stung the caterpillar three or four times. These thrusts were in the front part of the abdomen. The stinging and the malaxation do not kill the caterpillars, but leave them in a semi-paralyzed condition. In all of the nests that I opened, if they contained eggs or young larvæ, the caterpillars squirmed when exposed to air.

Visitors to the Colony.

During my week's sojourn with these wasps several species of insects visited the colony. A large species of assassin fly occasionally visited the scene. She would rest quietly on the ground in the midst of the colony and make her toilet. When a wasp approached she would become as rigid as a stone; but never did I see her make an attempt to capture one of the wasps.

Two species of dipterous parasites appeared. One fly (probably a species of *Hilarella*[1]) occasionally followed a laden wasp in from her hunt. Just before the wasp entered the nest she made movements as though attempting to oviposit on the caterpillar. Another parasitic fly occasionally rested on the ground at the mouth of a nest from the time the wasp entered with a caterpillar until she emerged. She would then enter the nest, remain a short time, and then leave.

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[1] The scientific names of all the visitors are not given; because, when they were common, the experiments required so much of my time that I could not capture them. On the afternoon that I set aside for capturing them no visitors appeared.

In excavating the nests of this species the burrows of two more mining Hymenoptera were found. One of the nests (Fig. 6, *A*) is an incompleted burrow of *Cerceris* sp.? I saw the wasp leave this nest. The other (Fig. 6, *E*) had a long vertical burrow; near the bottom of the shaft, on one side, were two cells. These cells made an angle of about forty-five degrees with the central shaft.

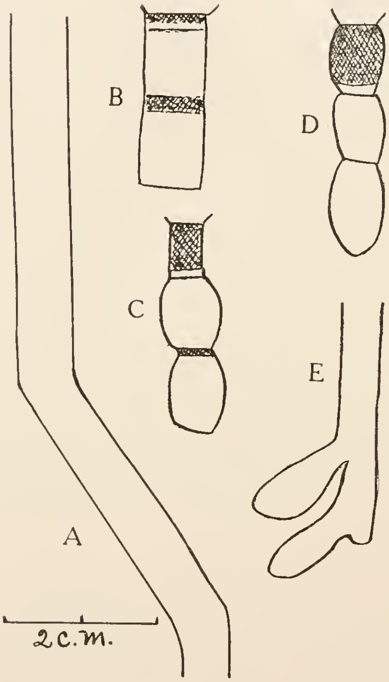

Fig. 6. Nests of certain wasps.
A, Burrow of *Cerceris* sp.? [Unfinished.]
B–D, nests of *Odynerus dorsalis* Fabr.
E, Burrow of an unknown visitor.

CONCLUSIONS.

1. The habitat of *Odynerus dorsalis* is a level, or practically level, tract of barren or almost barren land situated conveniently to a supply of water and of Hesperid caterpillars.

2. The nests are constructed in the ground; they are vertical and consist of one to three, usually two, cells, which are separated from each other by clay partitions. In rare cases the number of cells may be larger than this.

3. They occur in colonies of moderate size.

4. In order to work the clay it is wet with water obtained by the wasp from some near-by stream, or pond, or puddle.

5. In excavating the nest the wasp works the soil into balls and carries it out of the nest. Normally she flies with the pellet to a distance of from two to twenty-four inches, but if overhanging vegetation or other impediment renders flying with a burden unpleasant, she walks and deposits the pellets in one or more piles.

6. During the periods of excavating and of storing the nest the wasp is guided by landmarks; this is evidenced by the fact that any changes in the surface of the ground always serve to make it difficult for her to locate the nest.

7. The eggs are attached to the roof of the cell by means of a short thread. There seems to be no definite stage in the provisioning of the nest for the oviposition. At that time the wasp backs into the cell to lay and then walks out head first.

8. The Hesperid caterpillars with which she stores her nest are packed in until the cell is full. Since the cells are not all of the same size, the number of caterpillars in the cells varies. In all of the nests examined by me the lower cell is the larger.

9. In all of the cells examined by me the caterpillars were active enough to squirm when exposed to the light and the air.

10. If, during her absence to get water, pellets of mud or bits of weed stems are dropped into her nest, the wasp, on returning, removes them and deposits them on the ground near the nest. If paralyzed caterpillars are dropped into such a nest, under the same conditions, the wasp will fly with them to a distance of several yards.

11. If caterpillars captured by another wasp of the same species

are deposited in nests of wasps that are provisioning their nests, they will be removed and carried to a distance of several yards.

12. When slight changes are made in the surface of the ground near the nest, wasps returning home laden with caterpillars have difficulty in finding their homes, and sometimes enter the nests of other wasps.

13. In the midst of a colony of these wasps other mining Hymenoptera sometimes establish burrows.

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THE COLONY FOUNDING OF *ACANTHOMYOPS (DENDROLASIUS) FULIGINOSUS* LATR.

HORACE DONISTHORPE F.Z.S., F.E.S., ETC.

Putney, England.

Mons. R. Stumper ('20), having recently recorded the discovery of three isolated *fuliginosus* females in cells under stones, considers that these facts alone diminish the probability that the manner of starting colonies adopted by the *fuliginosus* is the dependent one, and that further investigation may prove this. We are personally of the opinion that the colony-founding habits of this ant are now well known and thoroughly established, not only by discoveries in the field, but by exhaustive experiments carried out in the laboratory. We propose to review the entire evidence on the subject in the order of date on which the various records and discoveries have been made; and to deal, therefore, with Mons. Stumper's remarks at the end of the paper.

Donisthorpe ('97) records that he found at Lymington a large colony of *A.* (*D.*) *fuliginosus* nesting in a hollow tree, and that a colony of *A.* (*Chthonolasius*) *flavus* was living in the same nest, both species coming in and going out together. With our present knowledge we know that it would be impossible for *fuliginosus* and *flavus* to live together, and that instead of the latter ant the species must have been *umbratus* or *mixtus*.

De Lannoy ('08) writes that in 1904 he found at Knoche-sur-Mèr in Belgium a few workers of *mixtus* living in a large colony of *fuliginosus,* and in 1906 he again found several similar colonies. He suggested as an explanation that the *fuliginosus* had stolen the larvæ and pupæ from a colony of *mixtus* which they had attacked, and that a few of the latter's brood, which had not been devoured, had been reared in the nest of the former ant.

Forel ('08) and Emery ('08), when commenting on de Lannoy's suggestion, expressed the view that it was a case similar to those in the *Formica* groups where the females have lost the power of founding their colonies unaided, and that a female *fuliginosus* after the marriage flight had been accepted into a nest of *mixtus*. After the natural or violent death of the *mixtus* queen, the brood of the *fuliginosus* queen had been brought up by the *mixtus* workers; the black ants gradually increasing and the yellow dying off little by little, until eventually a pure black colony was left.

Wasmann ('09) called attention to the ability of *fuliginosus* to form new colonies by sending off detachments of queens and workers after the manner of *Formica rufa*. He further accepted the interpretation given by Forel and Emery of de Lannoy's discoveries, and recalled the fact that he had himself on several occasions found mixed colonies of *fuliginosus* and *umbratus*. He hoped that future experiment would clear the matter up.

Crawley ('10) records that in 1898 he frequently found workers of a large bright yellow ant among the workers of a *fuliginosus* colony nesting in his house near Oxford. He thought at the time that the yellow individuals were workers of *flavus,* but is now satisfied that they were *umbratus*.

Donisthorpe ('10) points out that colonies of *fuliginosus* are often numerous in a district in which it occurs, and that it partly founds its colonies by branch nests. He mentions that Wasmann had frequently found *umbratus* nests at the foot of trees inhabited by *fuliginosus,* and that the queen of the latter had probably founded her colony in the nest of the former. He refers to Crawley's record of *umbratus* workers in a *fuliginosus* nest and to his own of 1897. He says he is now convinced that the species was really *umbratus;* he was not so well acquainted with our ants at the time, but he remembers distinctly thinking how large the "*flavus*" were.

Wheeler ('10), when describing an aberrant *Lasius* from Japan, discusses thoroughly the colony founding of *fuliginosus*. He writes: "Unlike the queens of the common *Lasius niger,* the queen of *fuliginosus*, after fecundation on her marriage flight, and on returning to the earth, is unable to start a colony unaided, and if prevented from rejoining the maternal colony, or a detachment of workers of her own species, has to seek out a colony of *L. umbratus* and have her young brought up by the workers of this ant. The *umbratus* queen must be killed either by her own workers or by the intrusive *fuliginosus* queen, so that the host species is destined eventually to die off and leave a pure and thriving *fuliginosus* colony." That this method of colony founding is actually adopted by *fuliginosus* queens is clearly indicated by the following observations which have been slowly accumulating during the past few years. He then proceeds to review the writings of Crawley, Donisthorpe, Emery, Forel, de Lannoy, and Wasmann; and gives some further illuminating and interesting remarks on the genus *Acanthomyops* (*Lasius*).

Donisthorpe ('11*a*), in a paper read before the Entomological Society of London on December 7, 1910, mentions briefly all that was known to date on the colony founding of *fuliginosus,* referring to the writings and views of all the above-mentioned myrmecologists. He further says that a worker of *umbratus* had been sent to him to name on September 20, 1900, by a Mr. Tuck, who had taken it in a *fuliginosus* nest situated in a horse-chestnut stump at Bury St. Edmunds. He then stated: "My friend Mr. Crawley and I intend to carry out experiments with *fuliginosus* queens and observation nests of *umbratus* next year."

Donisthorpe and Crawley ('11*b*), in a paper read before the Entomological Society of London on November 15, gave the results of the above-promised experiments, which they published in detail. These experiments were entirely successful, and proved without doubt that a female *fuliginosus* will be accepted and her brood be brought up by the *umbratus* workers. They reviewed all the previous discoveries and gave a complete list of the literature on the subject.

The same two authors ('13), in a voluminous paper on the founding of colonies by queen ants read at the Second International Congress of Entomology held at Oxford in 1912, gave a very complete account of the colony founding of this ant, including all experiments and discoveries, in detail, made up to date.

Donisthorpe ('13), in a paper on some remarkable associations between ants of different species read before the Lancashire and Cheshire Entomological Society, briefly referred to the colony founding of *fuliginosus* queens in nests of *umbratus* and *mixtus.*

Donisthorpe ('15*a*), in his book on "British Ants," gives a full account of the colony founding of this species. It seems as well to reproduce the whole of this account here, as it gives the main facts of the experiments by Crawley and himself referred to above (which would have taken up too much space to give in detail in this paper), and records further experiments. "Crawley and I have shown that the female (*fuliginosus*) does not lay till the year after impregnation, and we have proved her to be a temporary social parasite, as will shortly be seen. . . . *D. fuliginosa* often founds new colonies by branch nests, which accounts for the fact that many colonies are found in the districts where this ant occurs. After the marriage flight newly fertilized females are received back into the parent and other *fuliginosa* nests near by. Occasionally, however, deälated females are found wandering about in localities some distance from their nests. Forel records finding a number of deälated females on roads at Soleure on July 21, 1869, and Crawley found one at Oddington, near Oxford, about one hundred yards from a nest, and others at Esher in August, 1899, and in such cases as these the females would not be likely to be received back into their own nests. However, when isolated they display no desire to found a colony. Crawley and I have both kept in captivity newly fertilized females which get rid of their wings immediately after impregnation; and they never settle down, but endeavor to escape, and soon perish.

"Therefore, from the above facts alone, it seems doubtful whether the female of this species can found a colony unaided. But further observations in the field point to *umbratus* and *mixtus* as the host species of *fuliginosus*. In 1897 I found at Lymington a large colony of *fuliginosus* in a hollow tree, and *umbratus* was undoubtedly living with it, as workers of both species were going in and out of the same holes. Crawley in 1898 repeatedly found workers of *umbratus* walking unmolested with the workers of a large nest of *fuliginosus* established under his house near Oxford. In September, 1900, Tuck sent to me a worker of *umbratus* taken in a nest of *fuliginosus* at Bury St. Edmunds. In 1904 de Lannoy found at Knoche-sur-Mèr a few workers of *mixtus* in the midst of a large colony of *fuliginosus* which were on good terms with the workers of the latter, and in 1906 he again found workers of *mixtus* in several *fuliginosus* nests. Forel, Emery, Wasmann, Wheeler, and I commented on de Lannoy's observations, and expressed the opinion that the presence of these *mixtus* workers was due to the fact that fertile *fuliginosus* females had entered nests of the former species and been accepted. The queens of the *mixtus* had then died, or had been killed, either by their own workers or by the *fuliginosus* females, and the offspring of the latter were reared by the *mixtus* workers. In the course of time many of the latter had died off, and the few found in the nests were the survivors of the original *mixtus* colonies.

"Crawley and I determined to test this hypothesis by experiments on captive colonies. In July, 1910, a portion of a nest of *fuliginosus* was dug up at Darenth Wood, containing a quantity of workers, larvæ, males, and winged females, but no queen. The ants and brood were divided into two equal portions and each established in a four-chambered 'Janet' nest. (It may be here mentioned that this ant can not be kept in close confinement, but that if an observation nest be connected by long glass or india-rubber tubes to another plaster nest, or glass bowl, or some other contrivance in which their food is placed, the ants will thus obtain sufficient exercise.) During July all the males died and most of the females, with the exception of about twelve, which were found to be deälated. As some of these latter subsequently laid eggs, from which larvæ were reared, it is highly probable that mating had taken place inside the nests.

"In the beginning of December, 1910, a nest of *umbratus* without a queen was obtained at Weybridge and divided into two equal portions, which were established in 'Janet' nests. The first experiment was made on December 10, when one of the deälated female *fuliginosus* was placed in the light chamber of one of the *umbratus* nests. She immediately entered the most crowded chamber; one worker saluted her and another dragged her further by a mandible, but eventually she was attacked and killed before evening. On December 13 another deälated female was put into a small plaster nest with some of the workers from the same *umbratus* nest as in the former experiment. She was slightly attacked, but made no resistance, and endeavored to conciliate her assailants by stroking them with her antennæ. When a worker endeavors to bite at the waist of one of these females, she protects it by crossing her hind legs over her back, and when at the neck by pressing her head back close against the thorax. A few more workers were added, and on December 20 she was introduced with the workers into the *umbratus* nest. She was a little attacked by workers who had not seen her before, but the old workers protected her, getting between her and the others, and pulling them away by the leg, but very soon all hostility ceased, and she was evidently accepted. Many workers surrounded her, caressed and fed her, and all went well till April, when, a number of the workers having died off, some four hundred more were obtained from the Weybridge nest and introduced. These newcomers attacked the queen, though they were quite friendly with their sister workers, and as they persistently refused to accept her, she was removed and returned to her own nest on April 21. The *fuliginosus* workers were very excited at her appearance in their nest, and she was much pulled about, but eventually lost sight of amongst the crowds of ants. On July 23 a female with her gaster enormously dilated was noticed in the *fuliginosus* nest, with a large pile of eggs and surrounded by workers, which may possibly have been the female of the above experiment. In the next experiment a female *fuliginosus* was still more easily received into the other *umbratus* nest, and by December 16 she was completely accepted. On March 22, 1911, another *fuliginosus* was introduced into this nest and was immediately accepted without any hostility whatever, as was the case with two more which were introduced in April, but subsequently removed. The two queens first introduced in this experiment began to lay on March 17, 1911, for the first time, and these eggs hatched on August 9. In 1912 they began to lay on June 29, and laid more eggs than during the previous year, but nothing came of these. Some of the larvæ which hatched in August, 1911, were nearly full grown in the summer of 1912, but they remained in this condition until 1914. A larva first pupated on June 23, 1914, and several more subsequently, but none of them reached the perfect state. As these were from eggs laid in May, 1911, it will be seen that they took over three years to develop as far as the pupal stage!

"In 1913 I made a similar experiment with a nest of *mixto-umbratus* obtained at Weybridge on August 11, 1912, and subsequently strengthened with workers of *umbratus* from Wellington College. On September 14, 1913, two fertilized females, from the Oxshott nest before mentioned which had just removed their wings, were introduced into the *mixto-umbratus* nest, when they were very little attacked and completely accepted the same day. The one died on October 12, but the other has been treated by the workers as their queen, having been fed, cleaned, and caressed by them, and is still alive in this nest—she laid her first eggs on July 12, 1914. As the females of *fuliginosus* are only slightly larger than the workers, and as their fertility is delayed for so long a period, it is clear that they are unable to found colonies unaided; they are hyper-temporary social parasites, since, as will be seen presently, *umbratus* and *mixtus* are temporary social parasites of *niger* and *alienus*."

Donisthorpe ('15*b*) recorded the death on August 29, 1915, of the above-mentioned *fuliginosus* queen which had been accepted on August 11, 1912, into a colony of *mixto-umbratus*. The workers in this nest had all gradually died off, and a large number of fresh *umbratus* workers were obtained from Woking and put into the nest. These accepted the old *fuliginosus* female at once. She was very weak and died a few days afterwards, but not from injuries. The workers had never shown any animosity toward her, treating her from the first as their queen.

Donisthorpe ('16), having been left with the above-mentioned queenless *umbratus* nest, obtained a number of virgin *fuliginosus* females from a colony of that ant nesting in the ground under a broom bush at Weybridge. On September 3, 1915, he removed the wings from one of these females and introduced her into the observation nest containing the *umbratus* workers. She ran about among the *umbratus* workers, tapping them with her antennæ; she was not attacked and soon gained the last (dark, damp) chamber of the nest, which contained the bulk of the *umbratus*. She appeared to have been accepted at once and was saluted, cleaned, and fed by some of the workers. She was treated as their queen, and was not attacked until September 7, when, the nest having been left in the sun, some of the workers began to attack her and pull her about. The nest was placed in a cool, dark place, and by September 19 she was once more thoroughly accepted as queen; she has not been attacked since, and on December 19 she was surrounded by a large court of attendant ants. Wheeler first demonstrated that if the wings be removed from a virgin *Formica* female it causes her to behave as would a fertilized one. I have subsequently found this to be the case in all such experiments with *Formica* females. In the genus *Acanthomyops,* Crawley has recorded that the act of removing the wings from virgin *umbratus* females was far from arousing the instincts possessed by a fertilized queen. In the above-mentioned experiment with a virgin female of *fuliginosus,* however, the effect caused by removing the wings was to make her act undoubtedly as would a fertilized queen.

Donisthorpe ('17) continues the story of the virgin *fuliginosus* female mentioned above. By April 22, 1916, her gaster had commenced to swell, and on May 25 she laid a few eggs. On June 9 a larger packet of eggs was present, held up in one mass by several of the *umbratus* workers. On June 25 two larvæ had hatched, the eggs having taken over two months to develop. The eggs continued to hatch very slowly, and on November 28 some fifteen small larvæ were present. On January 28, 1917, ten medium-sized larvæ were counted, and the *fuliginosus* female was surrounded by a number of the *umbratus* workers.

Donisthorpe ('18) reports further concerning this *fuliginosus* female. On May 1, 1917, a fresh packet of eggs had been laid and the gaster of the female was considerably swollen. On May 22 there were present over 200 *umbratus* workers, the *fuliginosus* female, one packet of eggs, and 9 full-grown larvæ. May 24 some of the *umbratus* workers were covering the larvæ with bits of plaster, etc., to help them to spin their cocoons. Unfortunately during the author's absence in June and July the nest was allowed to get too dry, and on his return he found the eggs, larvæ, and pupæ had disappeared, and most of the *umbratus* workers had died. On August 18 a number of *Acanthomyops* (*Donisthorpea*) *niger* worker cocoons from Woking were introduced, and these were collected by the few remaining *umbratus* workers. The workers from the *niger* cocoons began to appear on August 26, and by October 13 all had emerged and were surrounding the *fuliginosus* queen. A number of fresh *umbratus* workers were brought home from Weybridge on October 16 and a few at a time were introduced into the nest to strengthen the colony. At first the *niger* workers killed them, and this went on till November, when a few were received. From then onwards four, five, and six *um-*

bratus workers were added every day, the *niger* workers no longer attacking them; neither did they attack the *niger* workers, nor the *fuliginosus* queen. The latter began to swell with eggs again on November 20. On December 31, 1917, there were present 60 *niger* workers and a large number of *umbratus* workers; the latter now surrounding the *fuliginosus* queen.

Donisthorpe ('19) mentions that in 1918 the *umbratus* workers in the above-mentioned nest started to kill the *niger* workers, all having been killed by May 17, 1918, when only one remained, which was killed before the end of the month. The *fuliginosus* queen gradually got very swollen again, and by May 27 she had laid a small bunch of eggs, which was held up by several *umbratus* workers. On June 16 two packets of eggs were present, but they were all eventually devoured by the *umbratus* workers. (The following is extracted from my notebook and has not been published before. The virgin female *fuliginosus* died in May, 1919, having lived in captivity for nearly four years. On April 25, 1919, she was very swollen with eggs, but almost dead, being held up by a number of the *umbratus* workers; and on May 1 she was quite dead, but still carried about by the workers until May 17, when her body was cut up. The *umbratus* workers then commenced to die off, so they were turned loose in the garden in June.) He further records that on July 17, 1918, he discovered in the New Forest a very interesting mixed colony of *A.* (*D.*) *fuliginosus* and *A.* (*C.*) *mixtus*, which consisted of about two thirds of the former workers to one third of the latter. The two species were walking along together in files on a fence near a railway bridge; they were quite friendly, tapping antennæ and saluting each other when they met on the tracks, as also when placed together in a small tube. The tracks led right down the brickwork of the bridge to the ground beside the line. It was really a beautiful sight, when the sun was shining, to see the jet black *fuliginosus* and yellow *mixtus* marching in files up and down the wall of the bridge and saluting each other when they met. As *mixtus* is very subterranean in its habits, it must have learned from the *fuliginosus* to march in files in the open. The tracks also led to and from a thick bramble grove growing by the side of a fence along the buttress of the bridge, and here the nest was evidently situated. He pointed out that here was a case where a *fuliginosus* female had evidently founded her colony in a nest of *mixtus*.

On May 29, 1919, Mr. H. M. Hallett discovered a mixed colony of *fuliginosus* and *mixtus* in a wood at Cwrt-yr-ala in Glamorganshire. The shoots from the stump of a felled oak were covered with Aphides, and all over the stump and shoots numbers of *fuliginosus* and *mixtus* workers occurred together. They were in the proportion of about 6 to 4.

Stumper ('20) briefly refers to de Lannoy's records and the views held on the subject by Emery, Forel, and Wasmann. He considers that three, in particular two, discoveries he made in the summer of 1917 at Neuenstadt speak against the general validity of their opinions. There he found two isolated *fuliginosus* queens "in their cells," situated under stones; but no brood was present. The third was under a stone which covered a nest of *mixtus*, but "her cell" did not communicate with the galleries of the latter. It will be seen that his knowledge of the literature on the subject does not appear to extend beyond 1909—at any rate, he entirely ignores everything that has been written by Crawley, Donisthorpe, and Wheeler! His latest discovery, of course, really confirms the fact that *fuliginosus* females found their colonies in *mixtus* nests. The female in question was no doubt only waiting for a suitable opportunity to enter the *mixtus* nest. She was probably "in quarantine," for the same reason that beetles of the genus *Atemeles* hang about a nest of *Formica* before they enter it after leaving one of *Myrmica*. It is only in observation nests that a *fuliginosus* queen has to put up with such a severe test as going straight into a nest of the host species from her own. After fertilization, should a female not be able to return to the parent colony, or enter a nest of another colony of the same species, she would have to wander about in search of a *mixtus* or *umbratus* nest, and would naturally hang about near by after she had discovered it. She would not only thus lose her own nest "aura," but in part acquire that of the other. As to Stumper's two first instances, it is possible that there may have been colonies of *umbratus* or *mixtus* in the near vicinity. Or after hunting about in vain for such these females may have crept under stones for shelter. It is not to be supposed that every female who finds herself stranded is successful in her search for colonies of her hosts. Finally, as we have already shown, *fuliginosus* females are not much larger than their workers, and do not possess large gasters supplied with plenty of fat to enable them to bring up their brood unaided, especially taking into consideration their long-delayed fertility. *Moreover, it has also been proved by experiment that they are unable to do so!*

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OBSERVATIONS ON GIGANTIOPS DESTRUCTOR FABRICIUS AND OTHER LEAPING ANTS.[1]

WILLIAM MORTON WHEELER.

In any study of the very exuberant ant-fauna of the Neotropical Region one can not fail to be impressed by the striking contrast between certain genera like *Eciton, Pseudomyrma, Solenopsis, Crematogaster, Cryptocercus, Azteca,* and *Camponotus,* each represented by a large number of variable species, and genera like *Paraponera, Acanthognathus, Daceton, Blepharidatta, Stegomyrmex,* and *Gigantiops,* each represented by a single, very stable species. Of course, such monotypic groups may be regarded either as very ancient, embracing during some former age many species of which only one has survived, or as single species which, after acquiring generic status in the remote past, have since undergone little or no modification. The individuals of a species representing a monotypic genus may be either very rare or local, mere relicts of a bygone age, or prominent and ubiquitous over larger geographical areas. This is true of such ants as *Paraponera clavata* Fabr. and *Gigantiops destructor,* which I have recently had abundant opportunity to study in the jungle about the Tropical Laboratory of the New York Zoölogical Society at Kartabo, British Guiana. As the latter species is the more imperfectly known, I have singled it out for special consideration.

The name *Gigantiops destructor* conjures up visions of a huge-eyed, insatiable monster, a kind of Cyclopean insect-jaguar. Fabricius, when he first described the insect in 1804 as *Formica destructor,* certainly knew nothing of its behavior and probably gave it what seemed to him an appropriate specific name for any ant measuring a centimeter in length. More than half a century later ('58) Frederick Smith received specimens taken by Bates at Ega, Brazil, and believed them to represent a new species which he described as *Formica solitaria.* The following note was appended to the description: "This is a very remarkable insect; for independent of the enormously developed eyes and produced clypeus, the palpi are elongated to half the length of the thorax, the maxillary are six-, and the labial four-jointed. Mr. Bates says: "This curious solitary ant is never seen by more than one at a time, prowling about fallen leaves, etc., in the forest; I have never seen its formicarium and, from its solitary habits, have no clue to guide me in looking for it." Perhaps Smith was confirmed in his choice of the specific name by the monastic or ascetic appearance of the insect, its somber black livery, relieved only by the golden-yellow tips of its antennæ, its long, emaciated limbs and its huge eyes, perpetually dilated as if in astonishment and chagrin at the indecent behavior of other insects.

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 177.

Two short notes, however, by later observers indicate that *Gigantiops* may be neither an insatiable assassin nor a humble anchorite, but a harmless and perhaps rather frivolous creature, that may have become permanently goggle-eyed through an age-long endeavor to enjoy to the full the riotous beauties of its environment. Emery ('93) was informed by Albert Schulz that the "Brazilian ant, *Gigantiops destructor* Fabr., which is distinguished by its enormous eyes, leaps from twig to twig, like the *Odontomachus hæmatodes* living in the same places," and Mann (1916) says: "In life this is one of the most attractive ants encountered. It lives always in the forest, where it forages either among the branches of trees or on the ground. The movements of the foraging worker are rapid, comparable to some of our species of *Cicindela*, and the bicolored antennæ are kept constantly in motion."

Roger ('63) was the first to throw Smith's *Formica solitaria* into the synonymy and to establish the peculiar genus *Gigantiops*. In more recent myrmecological literature mention of the insect recurs sporadically and at long intervals, showing that it was rarely seen in the many collections of South American ants examined by Mayr, Forel, Emery, Santschi, and others. Its known range, as indicated by the literature and by specimens in my collection, is as follows:

Brazil: Ega (Bates); Pará (E. Goeldi, ex coll. Forel); Maranhao (Ducke); Pará, Abuná, Porto Velho, and Madeira-Mamoré R. R. (W. M. Mann).

French Guiana (Jelski).

British Guiana: Kaieteur Falls, Tukheit, and Tumatumari (F. E. Lutz); Penal Settlement, Bartica District (W. Beebe); Kartabo and Kalacoon (Wheeler).

Peru: Callanga (Staudinger).

Bolivia: Rio Beni (L. Balzan).

These localities show that *Gigantiops* has a very limited range compared with many Neotropical ants, since it is confined to a strip of South America east of the Andes and extending from about 10° north to 10° south of the equator.[1]

Gigantiops (Fig. 1) is a common ant in the forested portions of British Guiana, preferring shady places rather free from undergrowth and spending most of its time on the ground, running over the dead leaves. It occurs singly, as stated by previous observers, and really belongs to a forest-floor ant-fauna comprising also *Neoponera apicalis* Latr., *obscuricornis* Emery and *commutata* Roger, *Mesoponera constricta* Mayr, *Pachycondyla crassinoda* Latreille and *harpax* Fabr., *Paraponera clavata* Fabr., and *Ectatomma quadridens* Fabr. Those who are interested in mimicry will observe that in its form, the dull black color of its body and yellow antennal tips, *Gigantiops* bears such a striking resemblance to *N. apicalis* and *obscuricornis* that the latter might be regarded as its models. Furthermore, these Ponerines sting very severely, whereas *Gigantiops* can be picked up with impunity. In Kartabo, nevertheless, the models are much less frequently seen than the mimic. This is interesting in connection with the observations of Mr. Tee Van, who finds that in the same region many of the mimetic butterflies are much more abundant than their putative Heliconid models. It would be a mistake to suppose that *Gigantiops* acts as if it derived any benefit from its striking resemblance to the stinging Ponerines. It greatly surpasses them in agility and when pursued will even leap several inches in a very graceful, cat-like manner. On the rather infrequent occasions when it climbs onto bushes and is running over their foliage it will, if disturbed, leap, without the slightest hesitation, to another leaf or even to the ground and make off with great alacrity. When two *Gigantiops* happen to meet face to face, they exhibit a peculiar play. After stroking each other's heads for a moment with the yellow tips of their antennæ, they move from side to side, precisely like two persons who meet on the sidewalk and try to prevent each other from passing.

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[1] I find a note by von Motschulsky in a letter published in his "Études Entomologiques" (1855) and referred to in the "Stettiner Entomologische Zeitung" (1859), which seems to apply to *Gigantiops*. Speaking of the insects which he observed at Obispo, Panama, he says: "I observed a lot of ants of diverse and bizarre form, among others one bearing the closest resemblance to a spider, especially to a *Salticus*, and as it also has the ability to leap, I have named it *Salticomorpha nigra*." There are two objections to accepting the name *Salticomorpha* as antedating *Gigantiops:* first, there is no record of this insect's having been taken in Central America or even in Colombia, and second, von Motschulsky may have seen a *Pseudomyrma gracilis* Fabr., which resembles a black Attid spider in form and color, and have mistaken its erratic movements for leaps. The well-known arachnologist, E. Simon (in Emery, "Voyage de M. E. Simon" (Dec., 1887–Avril, 1888). Formicides, Ann. Soc. Ent. France 1890, p. 65 *nota)* noticed that "all the species of the genus *Pseudomyrma* reproduce exactly the forms and colors of the spiders of the genus *Simonella* Peckh. (Attidæ) and the resemblance is equally striking in their gait." For the present it seems advisable, therefore, either to treat *Salticomorpha nigra* Motsch, as a *nomen nudum* or to include it with a query in the synonymy of *Gigantiops destructor* Fabr.

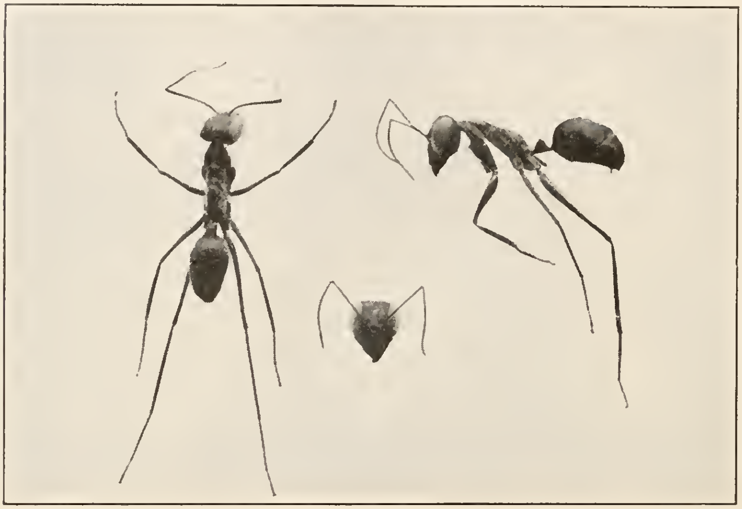

Fig. 1. *Gigantiops destructor* Fabr. Worker, about twice natural size; dorsal and lateral views and head from above.

On rare occasions *Gigantiops* may be seen carrying a termite worker or other small insect in its jaws, but even such individuals are not easily followed to their nests. Forel's placing of this ant in his tribe Œcophyllini, *i.e.*, with *Œcophylla smaragdina* Fabr., the well-known tree-ant of the Old World tropics, naturally led me to suppose that the nest must be in the trees, but this supposition, which has probably been shared by other myrmecologists, proves to be erroneous. On July 14, after much careful search and persistent following of single workers, my son Ralph succeeded in finding a nest in a partly decayed log only three or four inches in diameter lying on the ground at the edge of the Puruni trail and brought the portion inhabited by the ants into the laboratory. As soon as I began to dig into their nest the workers leaped out and made off, holding their larvæ in their mandibles. The colony comprised only fifty or sixty workers, which had been living in some large cavities made by *Passalus* or other wood-inhabiting beetles. In one of the chambers there were empty cocoons, showing that the pupæ of *Gigantiops* are not nude as in *Œcophylla*, its supposed nearest ally among the Formicinæ. I failed to secure the queen and believe she must have escaped unobserved among the workers. From these she differs merely in her somewhat larger size and slightly more voluminous thorax.

Notwithstanding careful search by my son and myself, ten days elapsed before I could again observe a *Gigantiops* nest. I found the second nest in a similar situation, in a partly decayed piece of a *Cecropia* trunk about a foot and a half long and three inches in diameter, lying loosely on the dead leaves in the shade of a bush. I noticed one of the workers timidly guarding a small hole and hastily retreating into it on my approach. The hole was plugged with cotton and the log carried back to the laboratory. In order that the ants might not elude me as on the previous occasion, I opened the log over a pail of water, but notwithstanding these precautions a few of the workers managed to escape. The entire colony, which was inhabiting one of the large internodal cavities so peculiar to *Cecropia,* was scarcely larger than the former colony, but contained more larvæ and several freshly spun worker cocoons.

No males were present and future attempts to find them and other colonies were unsuccessful.[1]

Two matters call for further discussion in connection with the foregoing observations, the taxonomic affinities of *Gigantiops destructor* and its *modus saltandi.* The generally accepted view of its taxonomic position can be traced to the various papers which Forel has published from time to time on the classification of the subfamily Formicinæ (= Camponotinæ Forel). In 1878 he placed the genus *Gigantiops* between *Opisthopsis* and *Œcophylla* in his first tribe of the subfamily. In his classification of 1893 he omitted all mention of *Gigantiops,* though he enumerated the various other genera of the subfamily. In 1912 he remodeled the classification and considerably augmented the number of tribes, to one of which, the Œcophyllini, he assigned the three genera *Gigantiops, Myrmecorhynchus,* and *Œcophylla.* The same arrangement is preserved in his paper of 1917. I endeavored to show in the same year that *Myrmecorhynchus* could not be retained among the Œcophyllini, but should probably constitute an independent tribe, the Myrmecorhynchini. The characters of the Œcophyllini, according to Forel, are the following: gizzard long and narrow, with straight calyx; clypeal fossa more or less distinct from the antennary fossa; antennæ inserted a little behind the frontal area, but near the anterior ends of the frontal carinæ. The gizzard characters are not peculiar to this tribe, but recur also in the Camponotini, and the remaining characters are decidedly weak, since they depend on slight differences in the proportions of the anterior portions of the head. When we compare *Gigantiops* with *Œcophylla* we are struck by the great differences in the structure of the larva, pupa and adult and in habits. That the habits of the two ants are totally different will be seen from a comparison of the observations above recorded with what we know of *Œcophylla,* and its various subspecies and varieties, which are arboreal ants inhabiting peculiar nests made of leaves and silk spun by their larvæ. Still it may be objected that such ethological peculiarities have little significance, since we have species of *Camponotus* that live in similar nests (*C. senex* Smith and *formiciformis* Forel) and others that live in the ground or in rotten logs (*C. maculatus* Fabr. and *herculeanus* DeGeer). Turning to morphological characters, which the taxonomist regards as much more reliable, we find that the only resemblances between *Gigantiops* and *Œcophylla* (apart from the shape of the gizzard which both share with *Camponotus*) are the shape of the clypeus, with its great, projecting lobe, the shape of the mandibles, and the feeble characters cited by Forel. There are great differences in the size of the eyes and claws, in the shape of the thorax and petiole of the worker, and in the size and shape of the thorax of the female, though the venation of the wings is similar. The larva of *Gigantiops* is like that of *Camponotus,* but very different from that of *Œcophylla,* and the pupa is inclosed in a cocoon. Probably the male *Gigantiops* will be found to exhibit some peculiar differences. Emery (*in litt.*) calls my attention to the singular fact that the tarsal claws of the male *Œcophylla* are almost completely atrophied. It would therefore be very interesting to know the condition of these organs in the corresponding sex of *Gigantiops.* The foregoing considerations seem to me to render it advisable to remove *Gigantiops* from Forel's tribe Œcophyllini and to provide an independent tribe for its accommodation. I find that Ashmead in 1905 had created such a tribe "Gigantiopini," though he included it in a subfamily Gesomyrmicinæ, with *Gesomyrmex* and *Myrmoteras,* genera which, in my opinion, are only remotely related to *Gigantiops.*

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[1] Dr. W. M. Mann, who has just returned with the Mulford Expedition from Bolivia, informs me that he found *Gigantiops* nesting under stones in the forests of the Rio Beni.

It is practically certain that *Gigantiops* is one of a number of ancient, large-eyed, active Formicinæ, once of very wide distribution, but now narrowly confined to the tropics. This group, which embraces also the genera *Œcophylla, Dimorphomyrmex, Gesomyrmex, Opisthopsis, Santschiella,* and *Myrmoteras,* represents merely the surviving specialized tips of diverging branches of a primitive stock. In regard to *Œcophylla, Gesomyrmex,* and *Dimorphomyrmex,* we are actually in possession of considerable paleontological information. Mayr ('68), Emery ('05), and I ('14) have recorded the occurrence of two species of each of these three genera in the Baltic amber, of Lower Oligocene age; Emery ('91) has recorded an *Œcophylla* and a species allied to *Gesomyrmex* (*Sicelomyrmex* Wheeler) from the Sicilian amber, which is referred to the Middle Miocene; Förster ('91) a species of *Œcophylla* from the Middle Oligocene of Alsace, and Heer ('49) and Mayr ('67) two species from the Lower Miocene of Croatia.[1] More recently Cockerell ('20) described a species of this genus from the Eocene of England, and he ('15) and Donisthorpe ('20) three species from the Middle Oligocene of the same country. The one, or possibly two, extant species of *Œcophylla* are now confined to the hottest portions of the Ethiopian, Indomalayan, and Papuan Regions. Similarly the few extant species of *Gesomyrmex* and *Dimorphomyrmex* are known to occur only in Borneo and the Philippines.[2] In the same regions and in Burma we find the four species of *Myrmoteras*. *Santschiella* is known only from a single specimen taken in the Belgian Congo, the species of the genus *Opisthopsis* are confined to the Australian and Papuan Regions, and the Neotropical Region possesses only one of these ancient large-eyed Formicines, *Gigantiops*. This, as we have seen, has a rather limited range and is in all probability a true tropical relict, originally developed in and since mainly confined to that portion of the ancient South American continent known as Archiguiana. All of the genera above mentioned are forest ants and most of them are arboreal, but, as we have seen, *Gigantiops* spends most of its time on the forest floor and nests in small, partly decayed logs. *Opisthopsis* nests under bark, in the ground or in earthen termitaria, and I may add that Dr. F. X. Williams, who took the types of *Myrmoteras williamsi* Wheeler in the Philippines, informs me that this ant nests in the soil. From what we know, therefore, of the living and extinct forms, we are justified in concluding that the large-eyed Formicinæ originated during the early Tertiary, or more probably during the Cretaceous, and that the extant forms have since undergone little or no modification, owing to the very stable ecological conditions in which they were able to survive. *Gigantiops,* in particular, may be said to have an even more remote origin than the archaic though highly specialized Neotropical vertebrates, such as the opossums, manatees, sloths, armadillos, ant-eaters and tapirs among mammals, or the ostriches and hoatzins among birds.

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[1] Since the completion of this paper Professor Cockerell ('21) has described a peculiar large-eyed ant from the Green River Eocene of Wyoming as *Eoformica eocenica*. It seems to belong to the subfamily Fomicinæ and resembles the Australian *Opisthopsis* in the shape of the head and the position of the prominent eyes.

[2] Since this paper was written I have published the description of a *Gesomyrmex* (*G. howardi*) from China.

The jumping or leaping habits of *Gigantiops* are so unusual that a more general account of this behavior as it occurs in various Formicidæ may not be out of place. There are two very different kinds of leaping ants, one which I shall call "retrosalient," which always leaps backward, and one that may be called "prosalient," because it always leaps forward. Some authors regard the former as not "leaping," in the proper sense of the term, probably because of the direction and because it is not performed by means of the legs. But such very abrupt displacements of the body are effected in so many different ways in different insects, as, *e.g.*, in Elaterid beetles, Lepismids, Collembolans, cheese-maggots, fruit fly and *Vermileo* larvæ, the extraordinary Coleopteran (?) cocoons described by Berlese ('20, p. 631), etc., that such words as "leaping" can hardly be avoided without pedantry. We even speak of fish or of a cataract "leaping."

Retrosalience has been repeatedly observed in two quite unrelated groups of ants, one embracing the Ponerine genera *Odontomachus* and *Anochetus*, the other the Myrmicine genus *Strumigenys*. By convergence both of these groups have developed very similar long, straight, and linear mandibles, inserted close together on the front of the head and furnished with large, abruptly incurved teeth at their tips. When excited these ants open their mandibles so widely that they stand out at right angles to the long axis of the head or are even directed slightly backwards. And if one of the insects comes in contact with a solid object in its path, it closes them so suddenly and with such force that they make an audible "click" and the insect is thrown backwards through the air to a distance of several inches. I have described this behavior in detail in *O. clarus* of Texas ('00). It has also been observed in *O. chelifer* of Brazil by Schupp (Wasmann, '92) and in the common tropicopolitan *O. hæmatoda* by Nietner ('58), Ferguson (Wroughton, '92), Forel, myself, and others.[1] The method of leaping in *Anochetus* is precisely similar, as shown by the observations of Wroughton ('92) on *A. sedillotii* var. *indicus* of India, and of Biró (Emery, '97) on a Papuan species. Among the Dacetonini it has been observed by Hetschko in the Brazilian *Strumigenys saliens* (Mayr, '93) and by Biró ('97) in *S. chyzeri* Emery ('97) of New Guinea. The worker of the latter species is able to leap backward to a distance of 20–25 cm., or about 100 to 150 times the length of its body, but this behavior is not exhibited by the female. Forel ('93) believed that the Neotropical *Acanthognathus ocellatus* Mayr and *Daceton armigerum* Latr. might be able to leap in the same manner. I have failed to observe the habit in the allied Australian ants of the genus *Orectognathus*. All the retrosalient ants, however, leap rather reluctantly and only under certain conditions, and the length of their leaps varies directly as the degree of solidity of the objects against which they happen to close their mandibles.

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[1] Borgmeier ('20) has recently described the similar habits of *O. affinis* in Brazil. "They strike their mandibles against the solid substratum and at the same moment leap 30 to 35 cm. vertically into the air."

Prosalience is exhibited by at least three very different groups of ants: certain bull-dog ants of the Australian genus *Myrmecia, Gigantiops,* and the extraordinary genus *Harpegnathos,* or *Drepanognathus,* as it was formerly designated, of the Indomalayan Region.

The leaping Myrmecias, popularly known as "jumpers" in Australia, comprise the members of Emery's subgenus *Pristomyrmecia* (*fulvipes* Rog., *mandibularis* Sm., and *piliventris* Sm.) and the smaller species of *Myrmecia* sens. str. allied to *nigrocincta* Sm. and *pilosula* Sm. I have frequently seen these ants jump distances varying from one to a few inches. When disturbed *M. nigrocincta* and *pilosula,* especially, present a ludicrous appearance as they bound out of their small mound nests in a series of short hops like Lilliputian cavalry galloping to battle. Examination of these ants reveals a structural peculiarity which has been overlooked by previous observers, namely, a distinct elongation and basal incrassation of their hind femora, as compared with the hind femora of the other nonsalient species of the genus. In Fig. 2 the hind femur of a worker *M. nigrocincta* (*c*) and that of a small worker of *M. sanguinea* of the same size (*d*) are drawn to the same scale. The greater length and volume of the femur of the former species shows a distinct approach to the conditions in the saltatory Orthoptera and is evidently to be interpreted as an arrangement for the accommodation of a more voluminous and therefore more efficient extensor, or abductor muscle in the hind leg. This morphological peculiarity, together with the leaping habit, seem to me to be sufficient to justify a separation of these jumping species from the remaining Myrmecias as a distinct subgenus, for which the name **Halmamyrmecia** subgen. nov. (with *pilosula* F. Smith as subgenotype) may be proposed.

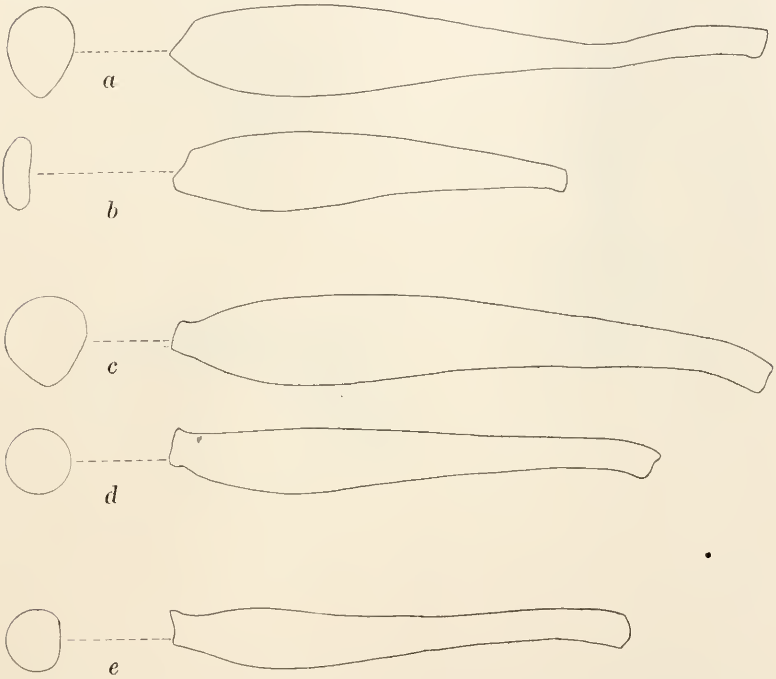

Fig. 2. Femora in profile and cross section of five species of ants drawn to the same scale. *a. Gigantiops destructor; b, Componotus castaneus americanus* Mayr; *c, Myrmecia nigrocincta* Smith; *d,* small *Myrmecia sanguinea* Smith; *e, Harpegnathos saltator* Jerdon.

In *Gigantiops* I find an even more pronounced elongation and basal incrassation of the hind femur than in *Pristomyrmecia* or *Halmamyrmecia.* As there is only one species of *Gigantiops,* I have compared its hind femur (Fig. 2*a*) with that of a non-leaping *Camponotus* worker of the same size (*b*). It will be seen that the difference in the length of the two femora is very pronounced, but that the difference in the basal incrassation is not so striking when the parts are seen from the side. The shapes of the cross-sections of the bases of the two femora, nevertheless, show that the *Gigantiops* femur is much more voluminous and therefore capable of furnishing attachment for a much larger and more powerful extensor muscle, as in the crickets and grasshoppers.

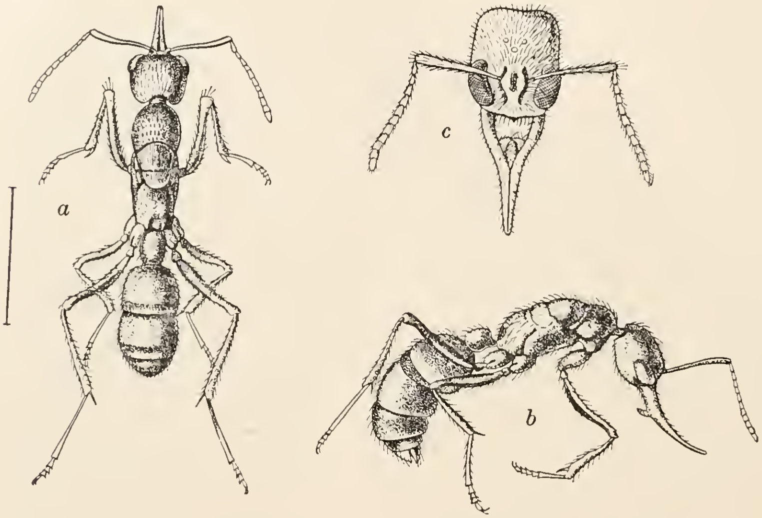

Fig. 3. Female of *Harpegnathos saltator* Jerdon (after Mayr.). *a,* dorsal view; *b,* lateral view; *c,* head of same from above.

Extraordinary feats of leaping are performed by the species of *Harpegnathos,* a genus confined to Indochina and the Philippines. In these ants (Fig. 3) the structure of the head is very singular, the eyes being very large, larger, in fact, than in any other Ponerine, and placed very far forward, and the mandibles are quite unlike those of any other known species. They are very long, separated at the base but approximated, arcuately curved upward, and gradually tapering toward their tips and finely serrate along their inner borders. Each is provided at the base with a large, flat, triangular tooth, projecting inward and somewhat downward and backward. In cabinet specimens the blades of the mandibles are applied to one another and the basal teeth overlap. The leaping habits of this insect have been observed by Lefèvre, Lewis ('82), Wroughton ('92), and Bingham ('03). Lefèvre states that it can make leaps of 20 to 25 cm., and Lewis saw it rise into the air to a height of 5 or 6 inches till exhausted, when its leap did not exceed an inch. Wroughton says: "The single specimen of the genus, which I have had the luck to find, made leaps of a foot or 18 inches with perfect ease, exactly like a grasshopper. I had much trouble in securing this specimen, and, when I succeeded, I found she could sting better than she could leap." Unfortunately we have no observations on the *modus saltandi* of this curious insect. Its hind legs are really very short and even thinner than those of many nonsalient ants as indicated in the figure of the hind femur of a worker *H. saltator* Jerdon (Fig. 2*e*). As this ant is nearly of the same size as the specimens selected for the other femora (*a–d*), and as the outline is drawn to the same scale, it will be evident that no such feats of leaping as described by Wroughton can be performed with such appendages. We must conclude, therefore, that the mandibles are employed for this purpose, but how they function is a matter of pure conjecture till the living insect can be carefully studied. It is conceivable that when about to leap the *Harpegnathos* opens her mandibles slightly till the two basal teeth just barely touch, and that she presses the tips of the mandibles against the ground so that their long, slender, and probably very elastic blades are more arcuately bent. We may suppose, moreover, that if the two teeth are suddenly permitted to slide over each other, with a concomitant sudden unbending of the mandibles, the insect would be precipitated forward much like a very elastic strip of metal or whalebone bent in an arc and suddenly released. It is less probable that the insect leaps by inserting the tips of the mandibles in the ground and bending them in the opposite direction, *i.e.,* by more nearly straightening them and then suddenly allowing them to return to their original curvature.[1]

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[1] Since this paragraph was written I find that Professor Forel (1921, p. 47) gives some notes on the method of leaping employed by *Harpegnathos.* He states, apparently on the authority of some correspondent in India or China, that this ant "fait des bonds formidables de plus d'un mêtre à l'aide de ses longues mandibules un peu recourbées en haut. La tête entière se recourbe sous le corps, se rejettant ensuite en avant, un peu à la manière du thorax de nos insectes d'Europe nommés taupins."

In conclusion, two interesting matters of a more speculative nature may be briefly discussed:

1. The various macrophthalmic, or large-eyed, tropical ants mentioned in this paper, namely, the Formicine genera *Gigantiops, Opisthopsis, Œcophylla, Gesomyrmex, Dimorphomyrmex, Santschiella* and *Myrmoteras,* the Ponerine genera *Myrmecia* and *Harpegnathos,* and I may add also the whole subfamily Pseudomyrminæ, constitute only a small percentage of the more than 10,000 extant species, subspecies, and varieties of Formicidæ. In the great majority of forms the eyes and ocelli of the female, and especially of the workers, have undergone considerable reduction in size or have entirely disappeared. In the male, however, which is the more conservative sex in ants, the eyes and ocelli are always large. These facts, together with what is now known of the ants of the Baltic amber, suggest that not later than Cretaceous time the females and workers were also all large-eyed, like the Scoliidoid wasps from which the Formicidæ are derived, and that they preserved this condition till after the social habit and a wingless worker caste had been evolved. During the Eocene and owing to the further development of the peculiar nesting and foraging habits, the females and workers became increasingly microphthalmic and anophthalmic till the small-eyed or blind condition became established in the majority of existing forms.

2. It will have been noticed that all the known prosalient ants have very large, convex, and minutely faceted eyes, and that all belong to archaic genera. I have already discussed the antiquity of *Gigantiops.* The Myrmecias are, I believe, justly regarded as the most primitive of existing ants, survivors, without more than specific diversification, from the early Eocene or the late Cretaceous. *Harpegnathos* includes only a few rare species of very restricted geographical range, evidently relicts on the verge of extinction. It would seem, therefore, that the leaping habit may have been much more general among the most ancient macrophthalmic Formicidæ, but had been abandoned as incompatible with a more highly developed social organization. This seems to be shown even within the genus *Myrmecia,* the larger and more dominant species of which no longer leap. That this habit should still persist, probably in a degenerate stage, in a few large-eyed forms,

may be due to the fact that ants endowed with unusual visual powers can retain such a habit with some impunity. Santschi ('11) and Brun ('14) have shown that vision is an essential factor in the homing behavior of ants that do not adhere very strictly to the topochemical trail made by themselves or their fellows from and to the nest. The leaping habit, if preserved and assiduously practiced in small-eyed ants, would, of course, often render it difficult or impossible to find the nest by means of the antennal sense alone.[1]

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[1] The mollusks of the genus *Pecten* are interesting in this connection, since, as is well known, they are able to leap considerable distances through the water by rapidly opening and closing the valves of the shell and have their mantle margins beset with large, highly specialized eyes.

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THE RELATION OF THE MADREPORITE TO THE PHYSIOLOGICAL ANTERIOR END IN THE TWENTY-RAYED STARFISH, PYCNOPODIA HELIANTHOIDES (STIMPSON).*

H. P. KJERSCHOW-AGERSBORG.

Ten Figures in the Text.

In a previous paper ('18) the writer recorded some facts concerning the bilateral tendencies and habits in *Pycnopodia helianthoides.* These were based on data gained from experimental studies and on others from observations on the behavior of the organism in its native environment. It was the writer's purpose at that time to follow the paper of 1918 with one on the physiology and histology, as well as other structural features of this species, and for this reason certain points were omitted from the paper mentioned above which might well have been included therein. Owing, however, to the writer's remoteness from the Pacific coast for the last five years and the lack of material on which to conclude this second work, it has been quite impossible for this additional contribution to appear sooner. During the last summer (1921) the writer was, however, so fortunate as to find opportunity to visit Puget Sound and gather some additional information on *Pycnopodia.* (It may be of interest to note in this connection that the transplantation of *Pycnopodia* from Bremerton to West Seattle was successful, which was shown by the fact that specimens of various sizes were found on September 2, 1921, on the piles under the docks at West Seattle, where about 12 specimens were planted in the spring of 1915, notwithstanding the difference in salinity at these two places. It was impossible, on account of lack of time, to determine whether the specimens found at West Seattle, 1921, were the same as those planted there in 1915, or whether they were the progeny of the latter. *Vide* Kjerschow-Agersborg, 1918.) From the additional data gathered this summer (1921) it is possible to conclude this paper, although in an abridged form.

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* Contributions from the Zoölogical Laboratory of the University of Illinois, No. 197.

Investigators do not agree in regard to the position of the madreporic plate in Asteroidea. Ludwig ('99), dealing with the genus *Asterias,* says:

"Falls wie es, trotz der nicht seltenen Ausnahmen die Regel ist, nur eine einzige Madreporenplatte vorhanden ist, liegt sie stets im vorderen linken interradius, wenn man den Seesterne bei abwärts gerichtetem Munde mit dem Interradius des Afters nach vorn stellt," p. 540.

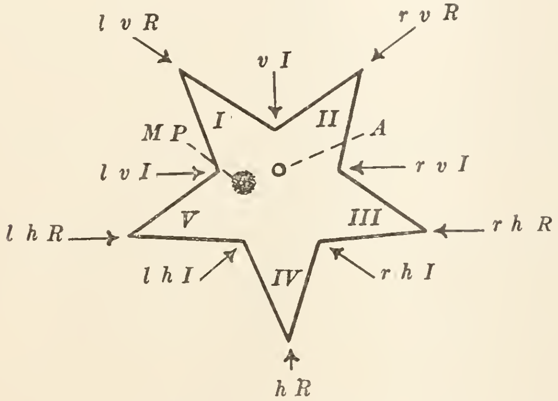

FIG. 1. A copy of Ludwig's figure of the asteroid groundplan, showing Ludwig's morphological "anterior" end. (P. 537.)

A, anus; *h.R,* posterior radius; *l.h.I,* left posterior interradius; *l.h.R,* left posterior radius; *l.v.I,* left anterior interradius; *l.v.R,* left anterior radius; *M.P,* madreporic plate: *r.h.I,* right posterior interradius; *r.h.R,* right posterior radius; *r.v.I,* right anterior interradius; *r.v.R,* right anterior radius; *v.I,* anterior interradius.

Agassiz ('77) claims that the madreporite is always in the suture of the terminal arm of the pentagon, which brings it opposite the third arm. This is also the view of Bury ('95). Rathbun ('87) says it is generally placed about midway between the center and the margin of the disk, and Sedgwick ('09) records that the madreporite varies in number in various species of starfish.

Delage and Hérouard ('03) say: "Elle est située dans la région apicale, tout près de la centro-dorsale, dans un interradius autre que celui qui porte l'anus. Le madréporite détermine une deuxième symétrie bilatérale, mais qui est altérée par l'anus, comme celle qui dépends de l'anus est altérée par le madréporite.

"Quand on regarde l'animal par la face dorsale, l'interradius madréporique vient après l'interradius anal, séparé de lui, d'un côté, dans le sens indirect (inverse de celui de la succession des heures sur un cadran), par un seul interradius, de l'autre par deux interradius," p. 2.

". . . quand on regarde l'animal par le pôle apical, l'anus est en arrière, le madréporite en avant et à droit," p. 3.

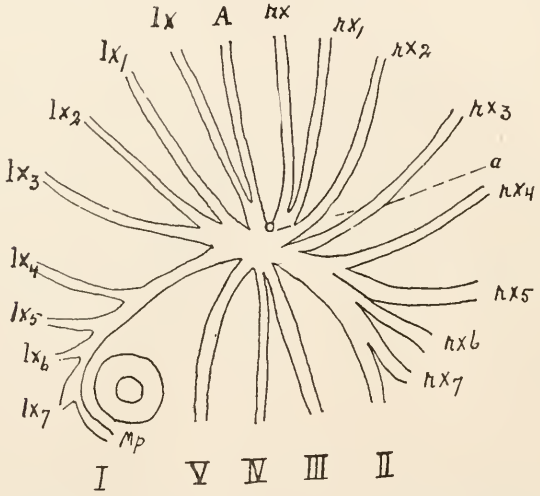

FIG. 2. Ritter's and Crocker's figure of the dorsal radial muscles of *Pycnopodia helianthoides*, showing the relation of the radial muscles to the asteroid groundplan, as represented in Fig. 1. *A*, the "anterior" ray; I-V, the "posterior" rays; rx–rx_7, rays added, on the right side, to the original six rays after metamorphosis; lx–lx_7, rays added to the left side; *a*, anus; *Mp*, madreporite.

Shipley ('93), MacBride ('96), and Lankester ('00) do not commit themselves in regard to the position of the madreporite, *i.e.*, as far as the starfish is concerned.

Ritter and Crocker ('00) in their work on *Pycnopodia* adopted Ludwig's and Agassiz's plan of numbering the radii of the typical five-rayed echinoderm. According to this, when the apical side is uppermost, counting from the madreporite clockwise, the rays are numbered from I.–V. These authors go on by saying: "From a study of the metamorphosis, Ludwig, '82, considers that the star should be oriented by a line running interradially between I. and II. and radially along IV., thus bringing the madreporite in the left anterior interradius" (Fig. 1).

"Before Ludwig was consulted, this orientation had been adopted for *Pycnopodia,* owing to the striking bilateral symmetry displayed in the young animals. This symmetry determined that the sixth ray is median anterior between I. and II., and by us has been called "*A*" (Figs. 4, 5). Ritter and Crocker's diagram (Figs. 4 and 5) agree with Ludwig's (Fig. 1). The relative position of ray "*A*," in *Pycnopodia,* according to Ritter and Crocker, to the five rays of the asteroid ground plan, is between the left and right anterior rays (Figs. 1, I.–II.; and 4, I.–II.); the axis of bilateral symmetry, or Ludwig's orientation line, lies along rays *A*–IV. (Fig.

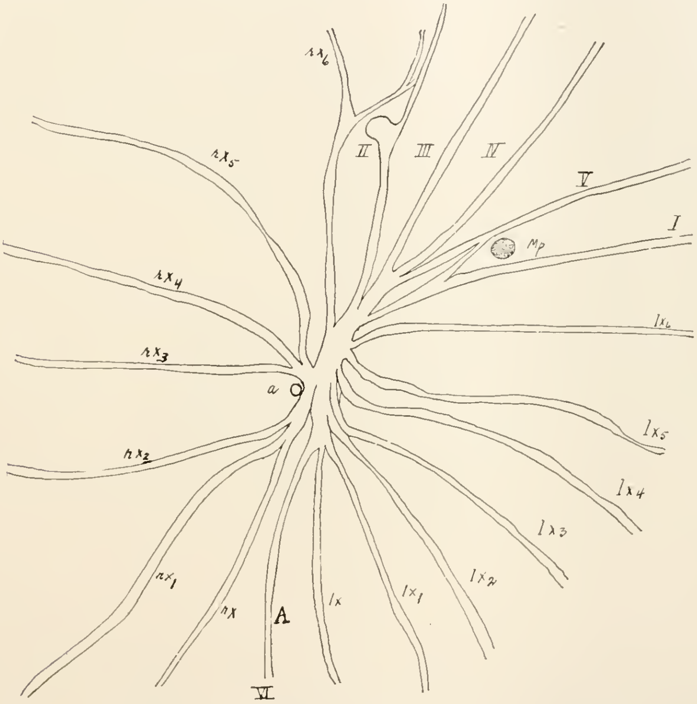

FIG. 3. A diagram of the dorsal radial muscles of *Pyncnopodia helianthoides* to show the relation of the primary and secondary rays to the physiological anterior end. (Rays I, lx_6, lx_5, lx_4. lx_3, correspond to I, V, IV, III, II (Fig. 2) respectively); rays I–V, rays of the physiological anterior end; these rays, and ray *A*, are, perhaps, the primary rays; rays lx–lx_6, the secondary rays of the left side; rx–rx_6, the secondary rays of the right side correspondingly; rays rx_6, and lx_6, the youngest rays. *a*, anus; *Mp*, madreporic plate. This sketch is made from the under side of the dorsal muscles.

4), or along a line drawn between vorderer Interradius-hinterer Radius (Fig. 1).

Delage and Hérouard claim that the relation of the madreporite is far from being precise, and their orientation of the asteroid plan corresponds to Ritter and Crocker's figures of young individuals, if they be inverted. This is true, however, only in a general sense for the madreporite which comes on the right side, but the anus is placed in the same relative position in either case. This relation is different in the adult. Here the madreporite is on the left anterior side between the rays I. and V. and actually corresponds to Ludwig's plan. But the so-called five primary rays of Ritter and Crocker, which, according to Ludwig ('99), correspond to 3–IV., 2–III., 1–II., 5–I., 4–V., respectively (Fig. 6), for the larva and the ground-plan of the common starfish, are not the posterior rays in adult life of *Pycnopodia,* however, but the anterior rays. This is easily seen in an actively moving individual, and may also be readily learned from the study of the interpolation zones which lie between rays II.–VI., VI.–I. It seems quite reasonable that the movements of *Pycnopodia* should be in the direction of, or along the axis of, the oldest rays, with the oldest rays as determiners of direction of movements. For, as I have shown before ('18), this

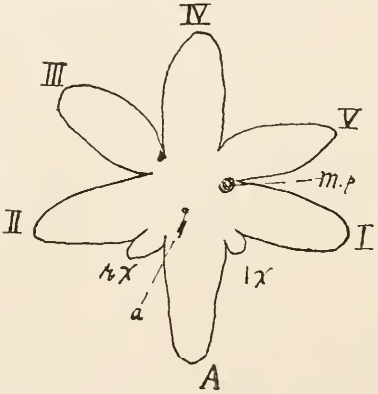

Fig. 4. Young individual, aboral view, in the eight-rayed stage (from Ritter and Crocker); the sketch is inverted to show the probably anterior rays (I–V) and their relation to the madreporite and the anus, *mp, a,* respectively. *A,* the posterior primary ray. (Compare this figure with Figs. 6, 7, 8, 9). The labeling is retained as in the original.

starfish does not only move with the same side all the time as anterior end, but its speed is considerably greater than that of the common star; its highest speed, *e.g.*, when moving to deeper water during the receding tide, was 122.5 cms. per minute. Opposite to the largest number of rays, in early post-larval life, is one ray, which is perhaps of the same age as the other primary rays, and with this single ray are young ones, varying in strength and age. As a matter of fact, it should be expected that if there are any distance receptors at all, which act as influencing factors on behavior, they would be present in the earliest developed tentacles. This seems to be probable in *Pycnopodia:* It always moves at right angles to the zones of radial growth; the anterior end in adult life, being always the side which possesses the fewest, but uniformly sized, rays anterior to the radial interpolation zones; *in young specimens the number of the large rays is greater for the anterior end*. In adults the number of the anterior rays is five; and the youngest of all rays is a pair, one on each side of rays II. and I.; the number of rays of the posterior end varies with the total number of rays. In living animals it is quite easy to distinguish between the ages of the rays. There is a gradual increase in size of the rays posteriorward from the interpolation zones, the youngest being, of course, found at the latter points. This seems to indicate that the so-called posterior end is the physiological anterior end.

Kjerschow-Agersborg ('18) demonstrated experimentally that out of 50 righting reactions of *Pycnopodia,* 46 were toward the anterior end with an average speed of about 57.1 sec. per normal turn. In this species a physiological anterior point is actually established (*vide ut supra et infra*), the anterior ray is ray IV.; the madreporite holds the relation as indicated by Ludwig, Agassiz, Ritter, and Crocker for the five-rayed starfish (Figs. 1, 4, 5, 6, 7).

It may be noted that in Ritter and Crocker's diagram (Fig. 2) the anus is in front of the base of the radial muscles, according to the interpretation of these writers; but by the establishment of the physiological anterior end the anus comes nearer the posterior end than before, while the madreporite is placed nearer the anterior end. This, however, does not change their relation to the respective rays, but the relation remains the same as before. That is, the madreporite is still in the interradius V.–I. of Ludwig. The position of the anus, in fact, corresponds, now, with Delage and Hérouard's asteroid plan. Figure 2 is copied from Ritter and Crocker ('00) and represents a diagram of the muscles of the aboral disk, seen from the inside, and as though looked down upon, showing the apical radial muscle bands, the madreporite, and the position of the anus close to the origin of the muscle of ray *A* (VI.). If ray *A* were the anterior end, then the anus would be anterior to the center of the dorsal side; but since ray *A* is, in fact, the posterior ray, the anus becomes placed posterior to the mid-dorsal point. Delage and Hérouard ('03) adopt Cuénot's plan ('91), with the madreporite in the interradius III.–II.; the anus in interradius II.–I., a little posterior of the mid-dorsal, but also a little to the right of the median line (Fig. 9). This seems to be an arbitrary matter of orienting the star. If the interradius I.–II. of Cuénot is placed as anterior end, the anus comes in the similar position as in Ludwig's plan: the madreporite is in interradius II.–III.; the anterior end is ray III. (Fig. 9). My drawing of the radial aboral muscles does not correspond at all with Ritter and Crocker's, in the point of orientation, and, in fact, also in the points relative to the general radiation of these muscles. I have compared the arrangement of the apical radial muscles in both large and small individuals having the same relative number of rays and

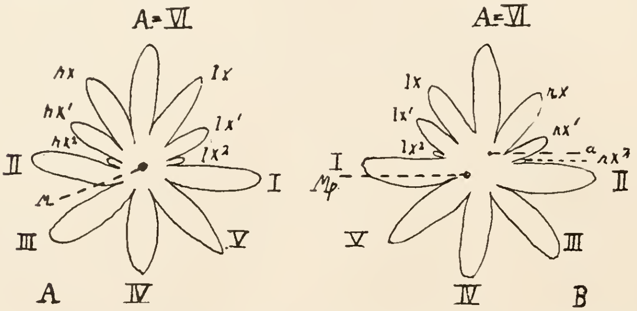

FIG. 5. Young individuals in the 12-rayed stage to show the interpolation zones: rx^2–lx^1. These figures are as per the original and show the reverse condition as in Fig. 4, which has been inverted to show the right relation of the madreporite to the true anterior end. *A*, oral view; *B*, aboral view. (From Ritter and Crocker.)

have found them to be exactly as represented in my sketch (Fig. 3). I can not accept Ritter and Crocker's orientation represented in their sketch (Fig. 2); but even if their interpretation should be correct, and indeed it may be for young individuals, *it does not hold for adults relative to their physiological anterior end.* Rays II., III., IV., V., I. represent the anterior end in respect to progressive movements of the starfish in its native environment (Fig. 3). The figure here is inverted, *i.e.,* the madreporite should face the reader on his left side; the sketch is made from the inner side of the aboral disk, looking down upon it. I am not concerned, at this time, about the controversy I may be led into relative to the difference between Ritter and Crocker's diagram and their interpretation and my diagram; I am only recording facts relative to the physiological anterior end in *Pycnopodia helianthoides* (Stimpson), and I am trying to adjust myself as far as possible with the accepted view in this field of morphology. In my opinion, the apical radial muscles of *Pycnopodia,* relative to their axial arrangement to the physiological anterior and posterior ends, fit in very well with the marked bilaterality of the adult, as I have recorded

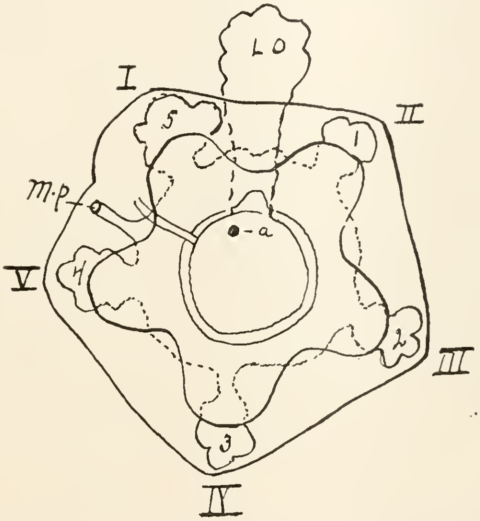

FIG. 6. Diagram of the asteroid groundplan of the metamorphosing common starfish. 1–5, radial vessels (larval); I–V, adult radial vessels; *a,* anus; *Lo,* larval lobe organ; *mp,* madreporite. (From Ritter and Crocker.)

elsewhere ('18). The radial symmetry is, indeed, superimposed.

Cole ('13) demonstrated with marked clearness, experimentally, what Cuénot and Delage and Hérouard had demonstrated morphologically, viz., that ray III. is the anterior ray. Cole, from his experiments on *Asterias forbesi,* constructs a line of bilateral symmetry with regard to his records; an approximately equal number of trials fall on each side (Fig. 8). A line drawn perpendicular to the line of bilateral symmetry, intersecting mid-dorsally, brings out of the total number of 499 trials: 347 ahead of it, *e.g.,* on the side which bears the madreporic plate; 152 behind, or to the side

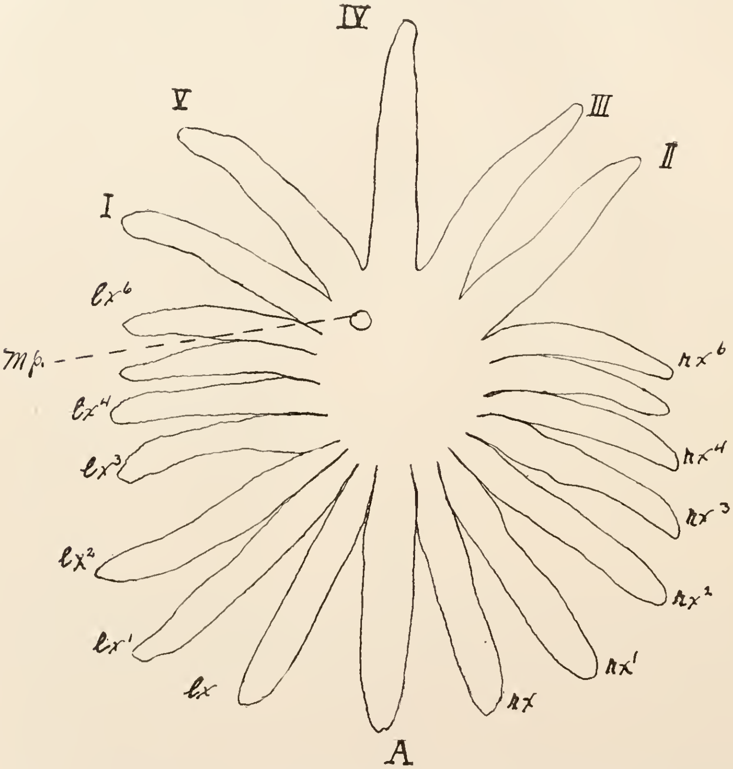

FIG. 7. Diagram of an adult individual of the twenty-rayed starfish to show the bilateral appearance in an individual actively moving about, and the relation of the madreporite (*mp,*) to the physiological anterior end (rays I–V); and of the anus (*a*) to the rest of the dorsal disk. *A,* primary posterior ray. rx–rx^6, the oldest and youngest right post-larval rays; lx–lx^6, oldest and youngest left post-larval rays.

which bears the anus. Ray IV. records 107 trials, ray III. 137, and ray II. 103 trials out of the total of 499; while rays V. and I. have only 74½ and 77½, respectively. This is, indeed, a very striking contrast to Ludwig's so-called "vorderer Interradius," *e.g.*, interradius I.–II., the *ipso facto* posterior end. If the physiological anterior rays of *Pycnopodia are in fact the morphological anterior rays,* as represented by Ritter and Crocker (although theirs must be inverted), then all facts agree. (Compare Figs. 2, 3, and 7.) In *Pycnopodia* the line of bilateral symmetry lies along the rays IV.–*A* (ray $A =$ VI.); this is explainable by the fact that five of the six primary rays become the anterior rays, with ray IV. in the lead, and rays III., II. on the left, and V., I. on the right (Fig. 7). The relation of the madreporite and of the anus to the dorso-radial muscles is then demonstrated (Figs. 2 and 3). Ray

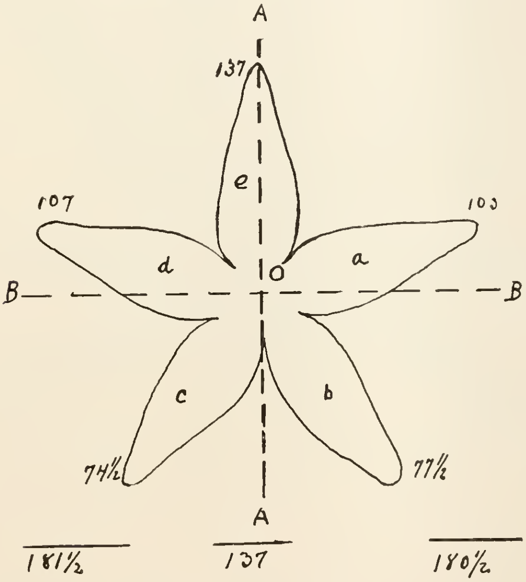

FIG. 8. Diagram to show the physiological anterior end of *Asterias forbesi,* and its relation to the madreporite. Line *AA,* plane of bilateral symmetry; *BB,* dissects the star into an anterior and posterior pole according to direction of locomotion. The *number* at the end of each ray indicates the exact number of attempts the star used that ray as "anterior" ray in locomotion; ray *e,* is seen to have the largest number in its favor. (Ray $e =$ III, Cuénot.) The sum of the trials indicates a "right" and "left" which correspond very well to a plan of bilateral symmetry. (From Cole.)

A (or VI.) is the median posterior ray (Fig. 7), while ray IV. is in the lead. Figs. 4 and 5 show the relation of the madreporite to the physiological anterior end in young specimens of *Pycnopodia;* the apical ray, *e.g.*, ray IV., is uppermost on the page (Fig. 4). In Fig. 5, *A* represents the oral view. Ritter and Crocker accepted as anterior end that pole of the star which corresponds to the larval lobe organ (Fig. 6, *Lo*), but if the physiological anterior end in *Pycnopodia* corresponds to the posterior end of the common starfish, then the larval lobe organ has nothing to do with it, at least not in *Pycnopodia.* It is, of course, an open question whether the anterior rays (II., III., IV., V., I., Fig. 3) correspond to the posterior rays of the same numbers in Ritter and Crocker's sketch (Fig. 2). If they do not, then the physiological anterior end in the adult *Pycnopodia* has become established independently of the factors as noted above. But this may not be the case. Of course, the common starfish may indeed move with any of its rays in the lead (Jennings, '07, p. 155); and "do the same thing, under the same conditions, in a number of different ways, and never do the same thing twice in exactly the same way" (Coe, ('12)). The anterior ray of these species of starfish (*Asterias forreri, A. for-*

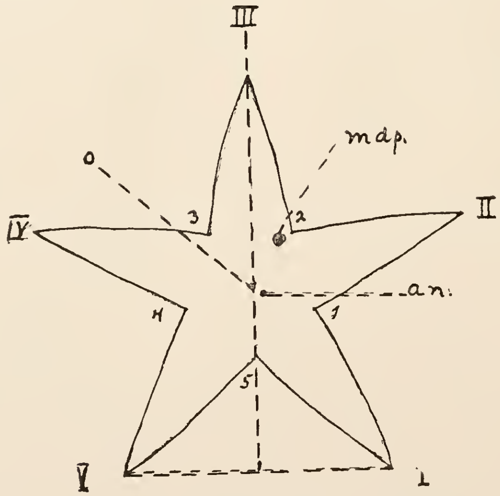

FIG. 9. Diagram to show the morphological groundplan of the common starfish. III, anterior end; *an*, anus; *mdp*, madreporite; *o*, morphological center; 1–5, interradii; I–V, radii. (From Delage and Hérouard.)

besi, and *A. vulgaris*) has not been easy to determine in the adult. Although Dr. Cole, as we have seen, comes nearest to establishing an anterior pole in the five-rayed starfish, *A. forbesi* (Fig. 8). Jennings ('07), in experiments on righting reactions, also found that: "Individual starfish do have a more or less permanent 'set' toward the use of a certain ray in pulling themselves over in the righting reactions." *A. forreri* uses the same ray (*e.g.*, ray *e, vide* Fig. 8) that *A. forbesi* uses as anterior pole. This anterior pole seems to be directly homologous with the anterior end in *Pycnopodia*, judging the size of the rays and their relation to the smaller ones in the latter.

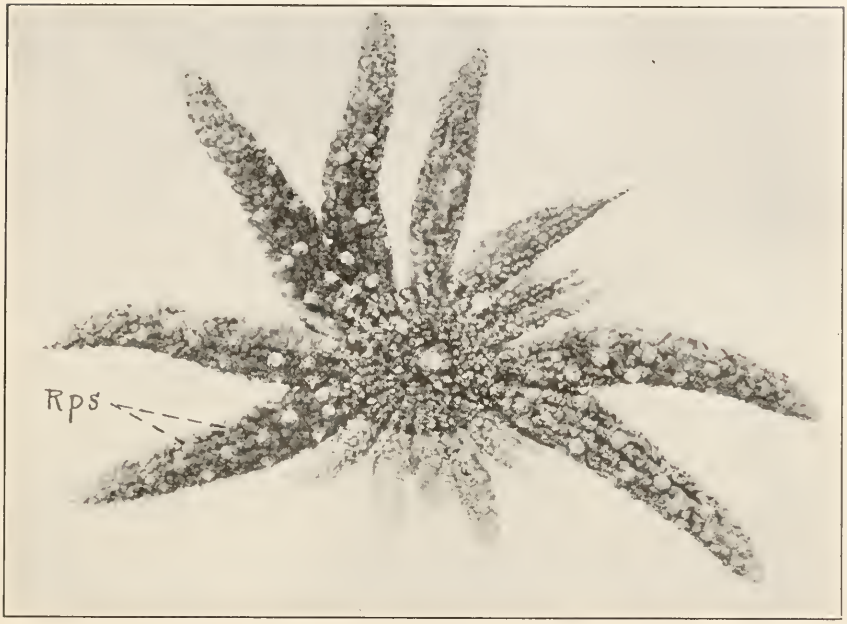

Fig. 10. Photograph of a small specimen of *Pycnopodia helianthoides* with 14 rays. Notice five rays on one side, and two on the opposite side, are regenerating from autotomy. *Rps,* respiratory papillæ. Natural size. Photo by author.

The number of madreporites, especially in multiradiate species, may vary: Cuénot ('91), Ludwig ('99), Delage et Hérouard ('03), Sedgwick ('09), Verrill ('14), Crozier ('21), and on that account the true position of the madreporite is difficult to determine. In a few cases I have found the madreporic plate present on the right anterior nearly opposite the base of ray V., but I have never found more than one in *Pycnopodia helianthoides*. Crozier ('21) records asexual reproduction in *Coscinasterias tenuispina* (Lamk.), its model number of rays in the adult being seven, and that, together with this method of autotomous multiplication, madreporites are also multiplied at separate points on the disk: "an assurance that portions of the body separated by autotomy will each be provided with a madreporite." He abandons the notion which he held at first, that "There is a physiological basis for the development of one or several madreporites according to the number of rays, *i.e.*, depending on the total water requirements of the locomotive organs." *Pycnopodia*, a much larger species (even though the ray lengths in the two species may be the same, *Pycnopodia* is larger in bulk than *Coscinasterias*, because it has about three times more rays than the latter), and with a locomotive speed about nine times greater than *Coscinasterias*, has usually only one madreporite. That is, it gets along with only one. Sometimes autotomy is practiced in *Pycnopodia* (Fig. 10), but not for the purpose as recorded by Crozier for *Coscinasterias*. Verrill ('14) reports a multimadreporic case for *Pycnopodia*, but he considers that to be abnormal. It seems, therefore, that while the anterior end in *Pycnopodia* is much the same as in the common starfish, the position of the madreporite on the dorsal disk may vary. But it is always nearer the physiological anterior end. Crozier also finds this to be true for *Coscinasterias*. The fact that *Pycnopodia* is bilaterally symmetric even after metamorphosis (no one has seen the larva of *Pycnopodia*, but it is assumed that it is bilaterally symmetric as in other echinoderms) may be a factor which aids it in the development of a locomotive speed greater than that of other starfish, and that it consequently "selects," by means of the law of least resistance, the oldest and most efficient pole as anterior end. This pole, judging from the relationship which the smallest rays bear to the physiological anterior end, is at right angles to the two interpolation zones, the zones of the least locomotive efficiency during the postembryonic development. Ludwig's asteroid ground-plan, applied to the ordinary starfish, does not hold completely for the twenty-rayed starfish; his "vorderer Interradius" becomes the "hinterer Interradius." The physiological anterior end corresponds more nearly to Cole's finding for *Asterias forbesi*, viz., that part in proximity to the madreporite is the anterior physiological end, the madreporite being on the left anterior side.

SUMMARY.

1. The physiological anterior end in *Pycnopodia helianthoides* corresponds to the posterior end of Ludwig's ground plan for *Asterias.* The anterior end possesses a relatively larger number of old (large) rays in early life than it does in late life. The new rays are added at two interpolation zones posterior to rays II., I.

2. The madreporite is, as a rule, on the left anterior side; it varies in position, but it is always found on the anterior part of the dorsal disk, *i.e.*, at the base of rays III., IV., or V. The so-called five primary rays (Ritter and Crocker), which, according to Ludwig, correspond to 3–IV., 2–III., 1–II., 5–I., 4–V., respectively, are not the posterior rays in adult life of *Pycnopodia*, but the anterior rays. Ray IV. is the anterior ray in adult life.

3. The establishment of the physiological anterior end in *Pycnopodia* shows that the madreporic plate is anterior to the mid-dorsal and the anus posterior; this arrangement agrees fairly well with Delage and Hérouard's plan for the five-rayed starfish. It also corresponds with Cuénot's plan for the common starfish, with Jennings's "more or less permanent 'set,'" and with Cole's "physiological anterior"; it is the reverse of Ludwig's asteroid ground-plan relative to the numbering of the rays.

ACKNOWLEDGMENTS.

The writer wishes here to express his indebtedness to Professor Trevor Kincaid, of the University of Washington (Seattle), for his ever-ready assistance and financial aid in the collection of the data for this and the previous paper (1918) on *Pycnopodia;* to Professors H. B. Ward and H. J. Van Cleave, of the University of Illinois, for criticizing the manuscript of this contribution.

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Vol. XLII. May, 1922. No. 5

BIOLOGICAL BULLETIN

RETINAL REFLEXES OF NARCOTIZED ANIMALS TO SUDDEN CHANGES OF INTENSITY OF ILLUMINATION.

A. T. CAMERON AND C. H. O'DONOGHUE.

(From the Marine Biological Station, Nanaimo, B. C., Canada.)

In March last, Cameron and Sedziak, working in Winnipeg, published a note[1] describing a peculiar reaction of the frog (*R. pipiens* from Illinois), narcotized with benzene, to sudden changes of illumination, a reaction noted incidentally in rehearsing a class experiment on the relative pharmacological effects of different hydrocarbons.

"Injection of 0.5 c.c. of pure benzene into the anterior lymph sac of a frog weighing about fifty grams produces distinct effects within a few minutes. The eyes close, movements become sluggish, when turned over the animal recovers its normal position with difficulty, and in most cases can no longer do so after ten minutes. The body often becomes characteristically arched. The usual reflexes gradually disappear. Breathing ceases. With the gradual disappearance of the conjunctival reflex the eye opens, the nictitating membrane is usually drawn down, and the 'retinal' reflex can be observed. Initially it would appear to be evoked by any rapid change in intensity of illumination. When the hand is passed suddenly between the frog's eye and the source of light, within one or two seconds the head is moved, and usually the hind limbs also. When a bright light (electric light at a distance of one foot) is suddenly switched on, the response follows. On switching the light off the response is more marked. Direction of light to and from the pupil only, the rest of the head being screened from light changes, evokes the response. Gradually the response to sudden increase of illumination ceases. The response to sudden decrease of illumination persists much longer. Cessation of stimulus therefore appears to be more powerful than application of stimulus. Ether anæsthesia abolishes the response to increase of illumination first.

"The maximum effect is reached at from twenty to twenty-five minutes after injection, and the reflex disappears in another twenty to sixty minutes, or occasionally after even a longer interval. Quality of light does not affect the result; both red and blue light evoke the reflex. Intensity of light appears to be the governing factor. A retina fatigued by the shining of a strong light upon it for some minutes does not call forth such an active response on switching off the light. There may be no movement, but after a minute or two the response can be again elicited by further light stimulation. The degree of response varies in different animals from a slight head movement to such vigorous movements of the hind limbs as would be excited by a strong electric current applied externally. After the reflex has disappeared electrical stimulation still produces active muscular responses. . . . The optimum dose of benzene appears to be 1.2 per cent. of the body weight. Two per cent. induces convulsions and death rapidly, and the retinal reflex can not be observed, while 0.7 per cent. may not produce any definite effect. . . . Similar changes have been observed with toluene, and to a less extent with phenol. Chlorbenzene and brombenzene give the reflex when injected in somewhat larger doses. The reflex could not be detected after injection of similar doses of xylene, aniline, nitrobenzene, benzyl alcohol, and pyridine."

At the same time Fröhlich and Kreidl published an account[2] of similar observations on the prawn *Palæmon squilla* F. found under stones at low tide. These observations were carried out on the island of Brioni in the Adriatic, and initially were accidentally made during experiments to determine the action of certain drugs on the heat-narcosis of these prawns. Fröhlich and Kreidl found that a similar reaction to illumination changes develops in these animals when immersed in sea-water containing camphor or phenol. A small arc-light was used for illumination. Thus for camphor:

"Immersion in camphor sea-water solution of concentration 1 : 16000 is fatal to *Palæmon* in from five to fifteen minutes. The toxic effects appear as twitches of the limbs and claws, convulsive strokes of the tail, while, when a number of animals are placed in the same vessel, they cling to each other in an entangled mass which is not easily separable. The convulsions are of such a nature that the animal clings fast to the nearest object, especially to the soft legs of its neighbors. If at this stage of camphor poisoning before permanent paralysis sets in the animal is removed rapidly to sea-water, the toxic effects just described—muscular twitchings and convulsions—disappear rapidly. *There remains, however, a pronounced photic over-excitability,* which is manifested by the animal making *very pronounced springs* when the light intensity is rapidly increased, *but on removal of the strong light, violent springs and tail-strokes. . . . The rise of reflex-excitability on sudden lessening of light-intensity was constant at the same phase of camphor action. . . .* There was no trace of a reflex increase for tactile stimuli, neither by shaking the vessel, nor by gently touching the head or tail."

Similar results were obtained with phenol in sea-water. A concentration of 1:2000 was rapidly fatal, one of 1:5000 less rapid in action. Removal to sea-water before complete paralysis permitted the same light reactions to be observed, especially that of removal of light. Strychnine, ammonia, and caffeine did not produce the effect.

Fröhlich and Kreidl point out that under natural conditions various animals react more strongly to shadow than to sudden increase of light intensity. Sarasin in 1887 (*Diadema setosum*)[3] and Uexküll in 1896 (*Centrospinus longispinus*)[4] noted that sea-urchins react to shadow but not to light with movements of the spines, and Hess, in 1915,[5] showed that shadow, but not light increase, produces in them rotating movements of certain flask-shaped structures. Franz (1919)[6] has shown the existence of the shadow reactions in snails. Fröhlich and Kreidl consider that the reaction is protective, and that the difference in the "cerebrally" poisoned and centrally irritable *Palæmon* is only quantitative.

In the experiments now to be described we have endeavored to link up the experiments of these workers with those of Cameron and Sedziak, since the phenomenon is obviously the same in the two series.

EXPERIMENTAL RESULTS.

Experiments have been carried out with benzene, phenol, camphor, and menthol. The benzene used was Merck's "highest purity, crystallizable," the phenol and menthol were commercial preparations. Two preparations of camphor were used: one, a Japanese commercial product, M. P. 174° C.; the second, a thrice resublimed preparation with M. P. 173.5° C. The latter gave somewhat more pronounced results. The menthol and camphor solutions were made by dissolving them in about twice their weight of absolute alcohol, and adding this to sea-water. The drug was precipitated in finely divided form and saturation of the sea-water followed rapidly. Tests with the same amount of alcohol in sea-water gave completely negative results. Various strengths of solution were used. These are indicated in brackets. The changes in illumination were effected by means of an acetylene lamp, which was suddenly shone on the vessel containing the animals under observation, and suddenly screened or removed. In the following account "light" signifies a sudden marked increase of illumination, "darkness" a sudden marked decrease. The animals were contained in large beakers, and sometimes in large porcelain evaporating basins.

A large number of marine species have been tested. These were selected largely with an idea of ascertaining in how wide a distribution of species the reflex could be obtained. Our choice was to some extent restricted, however, since it was limited to the species easily available.

The experiments are arranged in the order of the species tested.

1. *Cœlenterata. Hydromedusa. Gonionemus vertens.*—A common jelly-fish found amongst the eel-grass (*Zostera marina*) near the station at low tide.

Benzene (1 : 2000) produced no effect. The animal contracted normally after 15 hours. Light variations produced no discernible effect.

Camphor (resublimed, 1 : 16000) produced no effect. The animal was quiescent after two or three hours, but still contracted when touched after 18 hours. Light variations produced no discernible effects.

2. *Echinodermata. Echinoidea. Strongylocentrotus drobachiensis.*—Sea-urchins found on the under surface of the float on the station wharf.

Benzene (1 : 2000) produced an immediate immobility, and light changes ceased to produce any movement of spines. The animal was completely narcotized in six minutes.

Camphor (resublimed, 1 : 16000) produced the same result, and was fatal in less than eleven minutes.

Menthol (half-saturated) produced quiescence in three minutes. Light changes produced no discernible results.

3*a*. *Arthropoda. Crustacea. Decapoda.*

(*A*) *Hemigrapsus nudus.*—A shore crab found plentifully between tides under rocks at the station. When specimens were placed in sea-water under the experimental conditions, "darkness" produced a sudden stoppage of movement, and an occasional "flick," very slight.

Benzene (1 : 1500) produced narcosis in one or two minutes. Before this was complete the crab reacted to "light" by general activity—movement of chelæ and legs, and sometimes of the eye-stalk. "Darkness" produced no effect.

Benzene (1 : 3000). Two animals gave a "darkness" response, definite, but not much greater than normal, after one minute. After five minutes even touching them produced no response, and after ten minutes narcosis was complete.

Phenol (1 : 2000) was fatal to these crabs in from 30 to 40 minutes. Some minutes previously to death both "light" and "darkness" produced marked reflexes.

Camphor (1 : 16000) produced no effect. The crabs were normal after 6 hours' immersion. The resublimed camphor (1 : 16000) produced in a somewhat small specimen (7 grams) a "darkness" response in 10 minutes, and in a larger one in 40 minutes. In 45 minutes both gave marked responses, which continued for some time. After 15 hours' immersion slight responses could be elicited. It was doubtful that these were greater than normal. The animals were otherwise normal at that period.

(*B*) *Epialtus productus.*—A "kelp crab," a somewhat larger species, found amongst eel-grass near the station at low tide.

Benzene (1 : 2000). A large specimen immersed in this solution became more active. After 45 minutes activity had nearly ceased. The "darkness" reflex was present, but not marked. It was shown by sudden spasmodic movements of legs and chelæ. After a further 15 minutes a "light" reaction was doubtfully present; the "darkness" reaction was marked. The latter slowly decreased. After 70 minutes' immersion the crab scarcely responded to any stimulus. It was then removed to fresh sea-water and slowly recovered.

Camphor (1:16000). A smaller specimen showed a marked "darkness" reaction seven minutes after immersion. There was a general "flick" of legs and chelæ, but no coördination. "Light" produced no effect. After nine minutes' immersion the reaction was no longer given; the animal scarcely responded to any stimulus. Transferred to fresh sea-water, it did not recover.

Menthol (saturated solution). A similarly sized small specimen showed the "darkness" reaction after 12 minutes' immersion. The "light" response became evident after 22 minutes, and after 30 minutes both "light" and "darkness" responses were distinct. The animal commenced to struggle violently, shed both chelæ and three legs, and thereafter became scarcely responsive to any stimulus and (35 minutes) was evidently moribund.

(*C*) *Cancer productus.*—A fairly large crab found on the sea-bottom near the station wharf. Specimens placed in fresh sea-water under experimental conditions gave no noticeable response to changes of illumination. "Darkness" produced very occasionally slight twitches, shown chiefly by movements of the claws.

Benzene (1:1500). A medium-sized specimen (93 grams) was immersed. In six minutes a "darkness" reaction was evident. In 18 minutes both "light" and "darkness" responses were marked. The response consisted of spasmodic contractions of the legs and claws, so that the whole crab was raised. When placed on its back and tested, all the limbs suddenly contracted. The third maxilliped shut and the abdomen was brought up tightly against the carapace. After 32 minutes "light" and "darkness" responses were still evident, but slight, and on touching the animal little response was evoked. After 38 minutes the animal was apparently dead. It was removed to fresh sea-water, but did not recover.

Phenol (1 : 2000) was tested on a larger specimen weighing 140 grams. The "darkness" response was first noted after five minutes. After 18 minutes the "light" response was present, and the "darkness" response was much more marked. A second immediate "light" response produced a much greater reaction. The animal was by no means narcotized at this stage, tapping and touching still yielding marked responses. After 45 minutes the "darkness" response was still noticeable, but at the end of 70 minutes scarcely any responses were obtainable. The animal was removed to fresh sea-water, but did not recover.

Camphor (1 : 16000) was tested on a crab weighing 96 grams. The "darkness" response was just observable after 22 minutes. After 50 minutes "light" produced a response, "darkness" a much more marked one. After 77 minutes the animal remained on its back. It reacted strongly to both "light" and "darkness." The reaction was still evident after four hours and the animal was then by no means completely narcotized. After seven and one half hours only feeble responses were given and the animal was evidently moribund.

Menthol (saturated solution) was tested on a crab weighing 75 grams. The animal became very active immediately after immersion. "Darkness" produced a slight response after three minutes. After 10 minutes the reaction was very evident, but only with distinct pauses between each test. It continued, with gradual lessening, for three hours. No definite "light" response could be elicited. The animal appeared normal throughout with the exception of this response.

(*D*) *Calianassa californiensis.*—A large burrowing sand-shrimp found at low tide at Lock Bay on Gabriola Island, about three miles from the station.

Benzene (1 : 1500) produced a marked effect in 20 minutes. The shrimps remained on their backs with only occasional tail-twitches. After 45 minutes' immersion they were removed to fresh sea-water. They slowly recovered. During this period "light" produced definite tail movements. "Darkness" did not produce a definite effect.

Phenol (1 : 2000) produced complete narcosis in 17 minutes. The animals were transferred to fresh sea-water. Neither during narcosis nor during recovery were any "light" or "darkness" reactions observable.

Camphor (1:16000) produced no effects. After four hours' immersion the animals were quite normal.

(*E*) *Spirontocaris paludicola.*—A small shrimp found amongst the eel-grass at low tide.

Camphor (resublimed, 1:16000) produced narcosis in 12 minutes. The animals did not curl up. Transferred to sea-water, they slowly recovered. During this period a "light" reaction was doubtfully present. No "darkness" reaction was observed. A somewhat larger specimen was narcotized within one minute. During recovery after transference to fresh sea-water similar doubtful effects were obtained.

(*F*) *Hippolyte californiensis.*—Small shrimps found amongst eel-grass.

Benzene (1:1500) caused at first activity. The animals became sluggish and showed some tendency to cling together in chains (apparently a similar effect to that observed by Fröhlich and Kreidl with prawns in camphor). During this period no "light" and "darkness" responses were observed. After 20 minutes the animals were transferred to fresh sea-water. Within two or three minutes "light" produced a definite response, and "darkness" a much more marked one. These consisted of slight twitches throughout the body of the animal, jerks of antennæ and legs sometimes sufficient to move the animal its own length from its original position. Tapping or touching the animal at this period produced no effect. A second experiment with (1:2000) benzene produced almost complete narcosis in 14 minutes, and, after transference to fresh sea-water, positive "light" and "darkness" responses in four minutes.

Phenol (1:2000 and 1:4000) was fatal within one minute. Phenol (1:10000) was fatal in two or three minutes. No responses to "light" or "darkness" were observed.

Camphor (resublimed, 1:16000) produced a marked action. At first the animals gave marked springs, then curled up. During this period "light" and "darkness" reactions were absent. After four or five minutes the animals were transferred to sea-water. They immediately commenced to relax. Several gave distinct responses to "darkness" similar to those described for benzene. The reactions to "light" were less certain. Some animals were killed by five minutes' immersion.

Menthol (half-saturated solution) caused almost complete narcosis in 14 minutes, during which time there was some tendency to curl up and to cling together. Transferred to fresh sea-water, during the recovery period the animals appeared to give some "light" and "darkness" responses, though these were less certain than in benzene, and certainly less great.

3b. Arthropoda. Crustacea. Peracarida.

Amphipoda.—Camphor (1 : 16000) produced no effect during 14 minutes on some specimens of a species found on eel-grass.

Isopoda. Pentidotea wosnesenskii.—A small isopod found on eel-grass. Normal animals under the conditions of experiment showed little response to "light" and "darkness." Occasional "flicks" backward of head and antennæ occurred, but could not be related to illumination changes.

Benzene (1 : 2000) produced a definite effect in five minutes, the "darkness" response being distinct, that to "light" much less, but still observable. Tapping the vessel or the table on which it was resting produced the same sort of response. The reactions persisted for 30 minutes. They consisted of movements of the antennæ, "kicks" when the animals were on their backs, and, when on their legs, these were extended simultaneously so that the whole animal was lifted, and at the same time the opercula were "flicked." The responses gradually lessened with time. The best were obtained when the animal had been brightly illuminated for one or two minutes, and then the source of bright light suddenly removed. An immersion of from two and one half to three hours proved fatal.

Phenol (1 : 2000) gave similar "darkness" and tap responses after 20 minutes. These were not so marked as with benzene, and "light" responses were not observed.

Camphor (resublimed, 1 : 16000) produced no effect in eight hours, the animals appearing completely normal. A saturated solution produced no effect.

Menthol (half-saturated solution) produced definite "light" and "darkness" responses within 10 minutes. The animals were not so narcotized as with benzene. The response was evident as a flick back of head and antennæ. The animals ceased to respond after 90 minutes' immersion. They were transferred to fresh sea-water and during recovery gave similar but slighter responses to "darkness."

4. *Mollusca. Nudibranchia. Hermissenda opalescens.*—A specimen was found on eel-grass.

Camphor (resublimed, 1 : 16000) was tested on this specimen. After an hour there was some appearance of a reaction to "light," but none to "darkness." After 11 hours the animal was still just alive.

5*a*. *Vertebrata. Pisces.*

(*A*) *Cottoid* (*sp.*).—Small fish, too young for identification, about one inch long, caught in pools near low-water mark.

Benzene (1 : 1500) was fatal in three minutes. Benzene (1 : 3000) was fatal in less than 13 minutes. No responses to illumination changes could be detected. A (1 : 5000) solution was fatal to some animals in seven minutes. At this period in two cases a definite reaction (flick of body) was obtained with "light," but none was observed with "darkness."

Phenol (1 : 2000) was fatal to two of three animals in two minutes. The third was transferred to fresh sea-water and gave one or two doubtful responses to light changes.

Camphor (resublimed, 1 : 16000) was without effect. The fish were normal after three and one half hours.

(*B*) *Pholis ornatus.*—Blennids caught at low tide amongst the eel-grass.

Benzene (1 : 3000). Two small specimens, about three inches long, were almost narcotized in three minutes. They were transferred to fresh sea-water. No reaction to "light" was observed, but several definite responses to "darkness" were obtained. After 12 minutes both animals were dead.

Phenol (1 : 2000). Two similarly sized specimens were immersed and became narcotized in one minute. No definite light responses were observed. The animals were transferred to fresh sea-water, but did not recover. Two similar specimens were immersed in (1 : 4000) phenol. Definite "light" and "darkness" responses were given within one or two minutes of immersion.

The animals ceased to respond after six minutes, were transferred to fresh sea-water, but did not recover. A large blennid, apparently completely recovered from treatment with camphor, immersed in this solution, gave almost immediately a definite response to "darkness," but none to "light." It ceased to respond after five minutes.

Camphor (resublimed, 1 : 16000). One large specimen, about five inches long, gave a definite "light" and a more marked "darkness" response after 10 minutes' immersion. These responses consisted of a twitch of the tail and sometimes of the whole body. Tapping the vessel containing the animal induced a similar response. That to "light" ceased first. After 15 minutes' immersion the animal appeared completely unconscious. It was transferred to fresh water and recovered. It gave no definite responses during recovery. A still larger specimen immersed in this solution gave very definite responses within three minutes, those for "darkness" being much more marked. The reflex gradually disappeared. After 13 minutes breathing had stopped, but the "darkness" response could still be elicited. After 20 minutes no responses could be obtained to any stimulus. The animal was returned to fresh sea-water and recovered. Two small specimens which had been kept several hours in the laboratory were narcotized in about 10 minutes. No illumination responses were seen.

(*C*) *Siphostoma griscolineatum.*—A species of pipe-fish about six inches long caught amongst eel-grass at low tide.

Benzene (1 : 3000). Two animals gave a very positive "darkness" response after eight minutes' immersion. That to "light" was less. The response was indicated by a spasmodic jerk. It could be elicited during an additional 15 minutes. Both animals gave marked responses even after tap and tactile stimuli had ceased to produce much reaction, and after breathing had stopped.

Phenol (1 : 4000). A specimen, previously treated with camphor, and apparently completely recovered, gave, after 10 minutes' immersion, positive "light" responses and more marked "darkness" responses. A fresh specimen immersed in a (1 : 5000) solution gave slight responses after 10 minutes' immersion, which were more distinct for "darkness."

Camphor (resublimed, 1 : 16000). A specimen gave a response to "darkness" in eight minutes. In 12 minutes it appeared narcotized, was removed to fresh sea-water for five minutes, and then replaced in the camphor solution. Subsequently it gave very marked "darkness" responses and less marked "light" reactions, these continuing during 15 minutes. It was then transferred to fresh sea-water and slowly recovered. No definite responses could be detected during recovery.

Camphor (1 : 16000). A specimen, kept for two and one half hours in the laboratory, was immersed. It appeared completely narcotized within a few minutes. It was transferred to fresh sea-water, and then replaced in the camphor solution. After repeating this procedure again, it gave slight but definite "light" and "darkness" responses.

Menthol (half-saturated solution) produced in two specimens complete paralysis within a few minutes. The animals became absolutely limp, in marked contrast to the action of the other compounds used, which ultimately produced rigidity. They were transferred to fresh sea-water and slowly recovered. No illumination responses could be detected at any period of treatment. A saturated solution produced an immediate "limp" paralysis. A particularly large fish (eight inches long) immersed in a one fifth saturated solution showed no apparent effect of any kind in 45 minutes.

(*D*) *Gasterosteus williamsoni microcephalus.* — Californian stiklebacks, caught when free swimming near the station wharf. Under the conditions of experiment changes in intensity of illumination produced no effect on the normal fish.

Benzene (1 : 1500). Three specimens were tested. One, 24 mm. long, gave distinct positive reaction to "darkness" in four minutes. This consisted of flicks of the tail and forward movements. During constant illumination at this stage the animal was quiescent. After five and one half minutes the animal appeared moribund, and on removal to fresh sea-water it did not recover. "Light" did not produce definite responses at any period. A second animal of similar size was completely narcotized in less than two minutes. Some doubtful "darkness" responses were observed. The third, somewhat larger (33 mm.) and more active, gave "darkness" responses in from four to five minutes after immersion. After 14 minutes the animal was motionless and had ceased to respond even to direct touch. On removal to fresh sea-water it slowly recovered. During this period "darkness" frequently seemed to produce a backward and upward movement four or five times the length of the fish. This immediately ceased on switching on the light, the animal sinking.

5*b*. *Vertebrata. Amphibia. Rana pipiens.*—The results of Cameron and Sedziak for the frog under the action of benzene and phenol have already been quoted. In order to complete this record, the following experiments were carried out at the University of Manitoba:

Camphor (commercial preparation) was tested in two ways. (*a*) 0.5 c.c. of a 20 per cent. solution in absolute alcohol was injected into the anterior lymph sac of a frog weighing 60 grams. There resulted gradual onset of paralysis, the respiratory movements being the last retained. The animal died in about one and one half hours. Change of illumination produced no effect at any period. Since the immediate result of contact of the solution with body fluid is precipitation of finely divided camphor, with subsequent slow solution, no certain dosage in this experiment could be attempted. In a second frog injected with a smaller dose paralysis intervened sooner, and again no "light" and "darkness" effects could be elicited. (*b*) Two frogs were immersed in water saturated with camphor. There was a much slower onset of paralysis. One was almost completely paralyzed in three hours, the second slightly affected. On removal from water breathing recommenced, but the animals subsequently died. There was no evidence of reaction to light changes at any period.

Discussion of Results.

It is evident that the response of animals under a certain type of narcosis to illumination changes is widespread throughout marine species; this is probably true for land animals also. Our observations have been limited to species in which definite responses could easily be detected. More detailed study would probably reveal minuter changes, which, if employed, would show that the phenomenon is even more general. It is probable also that in certain cases a greater range of dilutions of the chemical compounds employed would yield more definite results; *e.g.*, our results for certain species of shrimps are inconclusive, and we have been unable to demonstrate "darkness" responses with narcotized sea-urchins, though these have been reported for the normal animal.

In addition to the normal "shadow" responses quoted in the introduction may be mentioned here the well-known reaction of frogs to shadow (immediate diving) and that of cryptobranchiate nudibranchs (retraction of the gill-plumes within the branchial collar). Similarly *Euglena viridis* sinks.

The response itself is obviously adaptive (*i.e.*, of survival value, and so likely to have been accentuated by selection), since under natural conditions a shadow suddenly falling on an animal implies the interposition of a solid object between it and the light—*i.e.*, a probable enemy. It is extremely well developed in the pelagic fishes, as we found to our cost when trying to catch them. This normal response becomes less marked in all animals when examined in the laboratory under experimental conditions.

The peculiarity of the response in the narcotized animal seems to consist in its accentuation, actually, or relatively through the abolition of volitional movements and other reflexes, so that its manifestation can be controlled experimentally.

The reaction is always definitely a reflex, though the animal may be by no means in a condition of complete narcosis. While cessation of a very intense illumination undoubtedly produces greater effects than cessation of a lesser degree of it, the sudden change is the controlling factor, and is quite effective when made from a brilliant to a dull but distinct light. In almost all cases it is necessary to allow the animal to rest in dull light for a minute or two between tests, as after repeated subjection to strong illumination the response ceases. This is obviously connected with fatigue due to photochemical changes of the retinal pigment present alike in vertebrates and arthropods. While undoubtedly the retina is the receptor of the stimulus in animals possessing it, the normal reactions of sea-urchins suggest that their color spots can function in a similar way.

Increase of illumination either produces no effect or one much smaller than a similar decrease. There were one or two exceptions to this general rule. Thus *Calianassa,* a burrowing shrimp, gave positive reactions with "light" and none with "darkness." It is interesting to note in passing that certain animals normally living or moving in darkness appear to give such a reversed reaction. The well-known responses of the earthworm and of *Mya arenaria* may be quoted. But the subject can not be pressed further without more experimental evidence.

In a large number of cases the animal also responds to mechanical stimulation such as tapping the table on which the containing vessel rests at a period at which the retinal reflex becomes evident. The type of response is somewhat similar to that evoked by illumination changes and is often not yielded by the conscious animal subjected to the same slight stimulus. It nearly always ceases to produce a response much earlier than the "darkness" stimulus.

The results that we have recorded above have some interest from the pharmacological viewpoint. The action of the drugs employed is by no means similar in higher species (cf. Cushny[7]).

Camphor, in the frog, depresses the brain, and later the spinal cord, the action being a descending paralysis. There is no excitement, the reflexes gradually disappear, and the animal lies completely paralyzed. In mammals convulsions, due to stimulation of the higher areas of the nervous axis, are usually produced, and gradually pass into depression, stupor, collapse, and death from cessation of respiration, the cerebral cortex, medulla, and cord being in turn paralyzed. The terminations of the motor nerves are paralyzed in the frog by large doses (which may account for our failure to elicit the retinal reflex with camphor), but not in mammals. Menthol produces a similar series of phenomena, but there is less tendency to convulsions.

Phenol, in the frog, first causes depression and loss of spontaneous movements, later augmented reflex excitability, and finally tonic convulsions (due to increased irritability of the spinal cord). Complete paralysis of the central nervous system supervenes. Similar symptoms are produced in mammals, but there is often no preliminary stage of depression. The peripheral nerves and muscles are not affected in mammals and scarcely, if at all, in the frog. Benzene is stated to be much less toxic than phenol, though when inhaled in large quantities it may give rise to similar symptoms. In some animals it produces violent and prolonged convulsions.

The differences of behavior exhibited by frogs and mammals to these drugs are shown by our results for the specific reflex that we have studied to be even greater when the observations are extended to include invertebrates. Although undoubtedly, as Cameron and Sedziak found for frogs, the degree of reaction differs markedly in different animals of the same species, and though our results were in some cases affected by the length of time that the animals were kept in the laboratory before they were tested, yet such marked variations are shown that a distinct idiosyncrasy of different species to the action of certain drugs is indicated. Thus camphor was little or non-toxic and did not reveal the reflex in cottoids and isopods; to the frog it was toxic, but the reflex could not be demonstrated; while with crabs, blennids, and pipe-fish marked responses to illumination changes were obtained with a varying degree of toxicity. Camphor was non-toxic for one species of shrimp, toxic but ineffective for a second, toxic and effective for a third; it gave marked reactions with the prawn employed by Fröhlich and Kreidl.

Quantitative comparison of the different drugs is difficult, not only on account of the different concentrations employed, but chiefly because of our ignorance of the rate of absorption of these drugs by different species and different sized specimens. The results for phenol and benzene can perhaps be contrasted most satisfactorily; it would seem that benzene is slightly less toxic, but it was of all the drugs employed the one most favorable for the production of the retinal reflex. It is to be noted that in at least one species (the isopods examined) for which camphor was non-toxic benzene was distinctly toxic.

It would seem that in most of the species studied benzene produces on the central nervous system an action distinctly comparable to the typical paralyzing action of phenol and camphor, and relatively much greater than that it produces in mammals.

Dr. Mary Rathbun, of the U. S. National Museum, kindly identified the species of shrimps employed, and Dr. B. W. Evermann, of the California Academy of Sciences, the blennids.

We desire to express our thanks to the Biological Board of Canada for the facilities placed at our disposal for the carrying out of this work.

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AN ANALYSIS OF THE SPAWNING HABITS AND SPAWNING STIMULI OF CHÆTOPLEURA APICULATA (SAY).

B. H. GRAVE,

Wabash College.

The phenomenon of periodicity in the spawning activities of animals has attracted wide attention since its most striking example was made known in a series of papers by Collin, Kraemer, Friedlander, Izuka, and Mayer ('97–'08). These papers give the results of careful studies of the spawning habits of the Palolo Worm and show that both the Atlantic and Pacific species shed their gametes during particular phases of the moon and tides. The time of their swarming is so definite that it may be predicted as certainly as an eclipse.

Since these papers were published a distinct periodicity in the time of spawning has been revealed for a number of animals, related not merely to season, but to the time of the month. Many animals have long been known to spawn regularly at a certain time of the day or night. This paper does not deal with the problem of such diurnal periodicity, but only with lunar periodicity. Most of the animals which are known to exhibit a periodicity of the latter category are annelids, and the degree of perfection of their periodicity varies with that which is just discernible, as in *Nereis dumerilii,* to that of the Atlantic palolo, *Eunice fucata,* in which the swarming is restricted to a certain phase of the moon once a year. This curious phenomenon is not restricted to animals, but is shown quite as perfectly by the brown alga, *Dictyota dichotoma,* as described by Williams ('05), Hoyt ('07), and Lewis ('10). Some other species of the Dictyotaceæ also show a similar periodicity in the time of freeing their gametes.

Periodicity in Chætopleura.

When an attempt was made, in the summer of 1919, to obtain the eggs of *Chætopleura apiculata* for embryological study, it was discovered that they could not be had at all times. They were obtained plentifully from July 12 to 20 and then spawning suddenly ceased. The writer naturally supposed that the end of the spawning season had been reached and that no more eggs could be expected that summer. However, experiments were continued at intervals of a few days, and at the approach of the next full moon eggs were again obtained. This time the period of spawning extended from August 8 to 11, inclusive. No eggs were obtained after the eleventh of August, although numerous attempts to induce further spawning were made. By referring to the table which follows, it will be seen that spawning began at the approach of full moon in July and continued thereafter for approximately one week (or until the third quarter), then ceased for a time to begin again in like manner at the time of the next full moon, thus indicating a distinct lunar periodicity.

Table.

Phase of moon.	Dates on which spawning occurred, summer of 1919.
Full moon	July 12.
	July 13.
	July 14.
	July 16.
Third quarter	July 20. Spawning active, apparently at maximum, many eggs laid by large proportion of females. Spawning began at 8.00 P.M. and continued until 2.00 A.M.
	August 8. Spawning occurred about 9.00 P.M. No eggs had been obtained during the preceding two weeks which included the new moon and first quarter.
Full moon	August 10.
	August 11.
	No eggs obtained after August 11, although several trials were made.

During the summers of 1920 and 1921 experiments were continued for the purpose of determining the limits of the spawning season and the relation of spawning activity to the cycles of the moon. As a result of these experiments the time limits of the spawning periods were found to be much less restricted than the data collected in 1919 indicated. In general, the data obtained during the last two years show that eggs may be had in small quantities at almost any time. By far the greatest spawning activity, however, comes at the approach of full moon and continues for ten days or two weeks. This more active spawning period includes two or three days preceding full moon and continues until after the third quarter, or with slight abatement until two or three days after new moon. In 1921 the diminution of egg-laying came promptly at new moon. The periods of excessive spawning activity are followed by periods of low activity during which spawning is practically suppressed. These periods of low spawning activity extend roughly from new moon until the approach of full moon.

More specifically stated, there were ten-day periods in each of the months of June, July, and August, during which spawning activity was remarkable for the abundance of eggs extruded, and there were other periods between each of these high points when spawning activity was diminished.

The low spawning periods were somewhat more distinct in 1921 than in 1920. In both years approximately sixty per cent. of the eggs obtained were spawned between full moon and the third quarter, and perhaps seventy-five per cent. during the periods beginning three days before full moon and extending to the next new moon.

The data obtained during the three years agree except that the spawning season of the last two years extended over longer periods, and that the lunar periodicity was less marked than in 1919, when spawning was confined to a few days in each month.

A further comparison of the results obtained during the three years is interesting in that the total number of eggs secured during 1920 was almost double that of 1921 and very far in excess of that of 1919. There is no apparent reason for this variation in egg production from year to year, but similar observations on other species of animals are common.

It may not be amiss in this connection to point out that Lillie and Just ('13) have found spawning activities in *Nereis limbata* which are quite similar to those of *Chætopleura,* but the periodicity of the two species may or may not be identical.

In what is to follow reasons are given for believing that the spawning periods of *Chætopleura,* under natural conditions, are more restricted than the results of the experiments carried out under laboratory conditions indicate.

Spawning under Laboratory Conditions.

It may be that the spawning habit and presumably other behavior of animals under laboratory conditions is not a reliable criterion for normal behavior in nature. This comes out most clearly when all of the factors which influence spawning under artificial conditions are considered.

It may almost be stated as a general rule that sexually mature marine animals are likely to shed their ripe eggs when placed under abnormal conditions. It would seem, therefore, to be a futile, or at least an unnecessary procedure, to attempt to duplicate normal conditions in the laboratory as a means of inducing spawning. For instance, in order to secure the eggs of the small lamellibranch *Cumingia,* it is only necessary to take them from the sand in which they live and expose them to the air for an hour or two, whereupon, when placed in a pan of quiet sea-water, they begin to spawn abundantly within half an hour. Eggs may be obtained in this manner at almost any time during the summer, and it is not uncommon for every female which is brought into the laboratory to spawn. It is not probable that many of these females would have spawned at these particular times if they had been left undisturbed in their normal situations.

Consider the case of the annelid, *Hydroides.* In order to cause them to spawn it is sufficient to break away their calcareous tubes and place them in sea water. Under these conditions they shed their sexual products within a few seconds. Dr. E. E. Just states that it is only necessary to pinch their tentacles or snip them off with scissors to induce spawning. They need not be removed from their tubes. It would appear, therefore, that the spawning of mature sexual products, in these cases, is influenced more by shock than by normal conditions. In fact, the more unnatural the conditions are made the better. However, after the shock has been applied, they require to be placed in a quiet situation, and presumably the more nearly it approaches normal conditions the better.

If these data have general significance, we may suppose that experiments which are performed in laboratories upon the behavior of animals, especially with reference to spawning, should be checked up as far as possible by observations of their habits under natural surroundings where the possibility of shock is eliminated.

The writer is presuming, therefore, that the spawning periods of *Chætopleura* are restricted to ten days in each of the summer months, extending approximately from full moon to the third quarter. This, however, is not set forth as a fact. If laboratory data are wholly trustworthy, the spawning periods extend from full moon to new moon with the qualifications noted in the text.

Conditions Affecting Spawning.

As a matter of convenience in the study of the spawning periods of *Chætopleura,* it was found most advantageous to remove the animals from the shells to which they normally cling and to keep them in shallow crystallization dishes.

It was soon discovered that they spawn only at night, usually beginning about 7.30 or 8 P.M. and continuing until 10.30 P.M. It is not uncommon for them to spawn at intervals throughout the night.

During the day the animals were kept aërated by means of running water, but toward evening they were washed free from sediment and placed in dishes on laboratory tables. They rarely spawned in running water, but the change to quiet water furnishes an effective stimulus to spawning. Under these conditions one or more males usually respond within fifteen minutes, females somewhat later. If allowed to remain in running water, neither males nor females respond, so far as has been observed. It is therefore evident that these animals are affected by currents of water, and one naturally suspects that they spawn under natural conditions, either at low or high tide when the water is slack. No convincing evidence that they spawn at the time of low or high tide was obtained from laboratory experiments.

Although these animals rarely spawn in water that is strongly agitated, they spawn readily in dishes which constantly receive a fresh supply of sea-water, provided the inflowing stream does not perceptibly disturb or agitate the water in the dish. This shows that spawning is not induced by the accumulation of carbon dioxide. The same conclusion may be drawn from the fact that spawning frequently begins within a few minutes after the animals have been placed in quiet water, and that it is practically over before the surrounding water is greatly affected by respiration. During the summer of 1920, at the height of the breeding season, a few females spawned in strongly agitated water. This occurred only once (July 8).

The number of eggs obtained from one female varies from about three hundred to three thousand, the average number being about sixteen hundred. As a rule only a few eggs are obtained during the first night after the animals have been collected. The greater number of females spawn the second night. A few spawn also during the third and fourth nights, but usually not to any considerable extent.

Spawning Stimuli.

Soon after being placed in quiet water the males begin to emit sperms in a double stream which soon become dispersed as clouds throughout the containing dish. The females respond more slowly after from one to three hours. One is therefore likely to suppose that something is expelled with the sperm which serves to stimulate the females to extrude their eggs.

This phenomenon was noted by M. M. Metcalf ('92) while studying two species of *Chiton* in aquaria. It was also described by Harold Heath ('05). F. R. Lillie and E. E. Just ('13) showed, without question, that in the case of *Nereis limbata* a substance is expelled with the sperm which is an effective stimulus to the female to shed her eggs promptly. In similar manner they found that the males will not shed their sperm except in the presence of a female which contains mature eggs or when placed in a dish of sea-water in which a mature female had been allowed to remain for an hour or more.

In order to test the assumption that this is also the case in *Chætopleura,* twenty mature individuals were washed thoroughly with fresh water and isolated in finger bowls of sea-water. During the night three of these females spawned and six males shed sperms in quantity, thus showing that neither males nor females derive any stimulus essential for spawning from the opposite sex. It is evident that the females were not induced to spawn by the presence of the spermatic fluid from the fact that none of the eggs initiated development. After some hours had elapsed these eggs were artificially fertilized and a considerable percentage of them went through cleavage stages, showing that they were normal, mature eggs, and that they had not previously been subjected to spermatozoa.

This experiment, first performed in 1920, was verified during the past summer.

Both sexes are apparently affected by the change from running to quiet water (tide changes), presumably also by the change in pressure between high and low tides (mechanical shock), and most certainly by moonlight.

It is evident that these factors have not been analyzed satisfactorily, but Mayer ('07 and '08), by actual controlled experiments, has contributed at least a first step toward a solution. The writer is of the impression that Scott's ('09) explanation, in the case of *Amphitrite,* that the periodic maturing of the sexual products is due to the warming effect of the sun at periods of especially low tides, can not be generally applied and is not applicable to *Chætopleura.*

The non-committal statement made by Lillie and Just ('13), that the maturing of the sexual elements of these animals is dependent upon phases of the moon, involving, through lunar tidal variations, rhythmical alterations of conditions of nutrition, is less open to criticism and is as exact as the known facts warrant at the present time.

Many interesting suggestions concerning the cause of periodicity in spawning have been made by various authors, but most of them are undoubtedly wide of the mark.

Friedlander ('98–'01), after reviewing various possibilities, finally admits that he is wholly in the dark and arrives at the conclusion that no theories yet proposed are adequate to explain periodicity in spawning.

Mayer ('07 and '08) states, upon the authority of experimental data, that moonlight is the effective cause, and that the tides are unnecessary but contributing causes.

Hemplemann ('11) believes that the moonlight, acting through a period of several days, is the stimulus to the maturing of the sexual products, and that it may also be the immediate cause of swarming.

A theory proposed by Brunelli and Schroener,[1] that the swarming has its origin in original accidental shocks to the nervous system of these worms, and that their swarming activities finally became spontaneous or hereditary in a form that was favorable to the propagation of the species, is probably true, but it explains nothing. That the habit of spawning periodically has become hereditary is shown by the fact that the animals under discussion will spawn at the proper time even when the normal stimuli are altered or absent. See Mayer ('07).

Most of these theories are based upon abstract reasoning and not upon experimental data. It is apparent that there is something yet to be done before we can understand this curious phenomenon and its relation to the moon. That this habit has become more or less hereditary is shown by several authors. However, it must have originated in relation to external stimuli which are felt periodically. The cause of this type of periodicity must be fundamentally the same in all cases, although it is exhibited in a variety of ways, and at different times relative to the phases of the moon.

Experiments on Chætopleura.

Summer, 1920.

I.

June 20–23:
20 Chitons collected June 20.
1st night, no eggs.
2d night, no eggs.
3d night, no eggs.
* First Quarter, June 23.

II.

June 24–27:
30 Chitons collected June 24.
1st night, no eggs, some spermatozoa.
2d night, no eggs.
3d night, no eggs.

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* It is due to the last two authors mentioned to state that the writer has read only quotations from their papers.

III.

June 27–30:

15 Chitons collected June 27.

1st night, four females laid eggs; spermatozoa abundant.

2d night, two or three females spawned lightly.

3d night, two or three females spawned lightly.

IV.

June 28–30:

100 Chitons collected June 28.

1st night, no eggs.

2d night, great quantities of eggs from 12 to 15 females; spermatozoa abundant; eggs laid 9–12 P.M.

3d night, two females spawned in quantity; a few eggs from several others.

4th night, two specimens spawned.

5th night, a few still spawned.

Note.—Nearly all spawned second night. Spawning is nearly always on 1st or 2d night after collecting. Spermatozoa appear first in every case observed.

V.

* Full moon, July 1.

July 2–3:

20 Chitons collected July 2.

1st night, two spawned, one of these about 10 P.M.

2d night, two spawned; spermatozoa shed in abundance as soon as animals were placed in quiet water.

VI.

July 3–4:

158 Chitons collected July 3. Placed 25 per dish.

1st night, two spawned.

2d night, about 20 females spawned, and about 30 or more males shed spermatozoa. (This group continued to spawn freely for five nights.)

VII.

July 6–8:

100 Chitons collected July 6.

1st night, several specimens spawned about 10 P.M., and a few more after 1 A.M.

2d day, several females spawned between 9 and 10 A.M., July 8, and one or two continued at intervals during the afternoon; spermatozoa also abundant. Beginning about 5 P.M., many females (35–40) spawned in great abundance, laying 2,000–2,500 eggs each.

Note.—July 8 seemed to be the height of the spawning season. Spawning occurred day and night, practically all night and more or less during the entire day. Spawning during the day not seen before.

* Last Quarter, July 8.

Note.—Experiments so far show that females spawn at the approach of full moon, a few days before. Spermatozoa are shed for longer periods and more readily than the eggs. Spermatozoa appear in clouds very soon after animals are placed in quite water, 5–10 minutes, and sometimes continue nearly all night. Females sometimes spawn the first night after collecting, but more abundantly the second night.

VIII.

July 11–14:

100 specimens collected July 11.

1st night, several specimens spawned.

2d night, no eggs.

3d night, several specimens spawned.

4th night, several specimens spawned.

Note.—This group was not well cared for, because attention was necessarily diverted to other matters. There was considerable if not abundant spawning, although not comparable to that of July 8.

* New moon, July 15.

IX.

July 17–18:

100 specimens collected July 17.

1st night, two specimens spawned.

2d night, several specimens spawned in quantity, 7–10 P.M.

* First Quarter, July 22.

X.

July 22–25:

100 specimens collected July 22.

1st night, three spawned about one half usual amount of eggs.

2d night, about six specimens spawned in quantity, one half to two thirds maximum.

3d night, two specimens spawned lightly and a few eggs from others.

4th night, none spawned.

XI.

July 28–30:

75 specimens collected July 28.

1st night, one spawned; a few eggs from a second.

2d night, about ten specimens spawned in great abundance, some about three thousand eggs.

* Full moon, July 30.

XII.

August 4–6:

75 specimens collected August 4.

1st night, very little spawning.

2d night, about eight spawned in abundance.

XIII.

* Last Quarter, August 7.

August 7, experiment worthless. Chitons suffocated by standing without water.

XIV.

August 10–11:

120 specimens collected August 10. Good lot.

1st night, no eggs.

2d night, three specimens spawned 8–10 P.M.; almost none after 10 P.M.

* New moon, August 13.

XV.

August 20–22:

112 specimens collected August 20.

1st night, no eggs and no spermatozoa.

2d night, one female spawned lightly (300 eggs), also twenty eggs from another, and nine from a third.

3d night, one female spawned average number of eggs (1,500), two others about 300 each.

XVI.

August 24–26:

94 specimens collected August 24.

1st night, no eggs, no spermatozoa.

2d night, two males emitted clouds of spermatozoa; one female spawned good bunch of eggs, a second thirty-two eggs.

3d night, three females spawned lightly (100 to 300 eggs each).

Remark.—Very light spawning since new moon.

* Full moon, August 29.

XVII.

August 28–31:

30 Chitons collected August 28. Left in supply department in insufficient amount of water until 10 P.M. (Fault of collectors.)

1st night, experiment set at 10 P.M.; no eggs and no spermatozoa.

2d night, two females spawned lightly and one male emitted spermatozoa.

3d night, two females spawned maximum quantity of eggs, 2,500 each.

4th night, no eggs.

Note.—The last experiment is more or less untrustworthy, because the chitons were partially suffocated by improper treatment after collecting. The group, however, shows a distinct revival of spawning in that thirty chitons spawned many times more eggs than the larger group of the preceding experiments.

EXPERIMENTS, 1921.

Experiments on Chætopleura. Summer of 1921.

* Full moon, June 20.

I.

Experiment 1, June 24–26.

1st night, of twenty-five large chitons collected June 24, three and possibly four spawned in fair quantity, first night, perhaps 50–60 per cent. of maximum; none spawned before 10 P.M.

2d night, spawning began at 10.30 P.M.; probably over before midnight, as indicated by development of embryos next morning; five females out of twenty-five animals spawned one half to two thirds maximum; good quantity.

Remark.—Experience of last year has shown that most of the spawning is done the second night after collecting. Therefore, it is best to consider only 2d night in studying spawning habits, or else compare experiments as a whole.

* Last Quarter, June 28.

II.

Experiment 2, June 30–July 2.

30 specimens collected June 30.

1st night, no females spawned; a few males shed spermatozoa (2 or 3).

2d night, three females spawned about one half maximum.

3d night, two females spawned in considerable quantity, a third one lightly.

Remark.—Impression of good spawning activity during the last week in June. Plenty of eggs for embryological study. Isolated specimens also spawned readily and in quantity.

III.

Experiment 3, July 2–5.

60 specimens collected Saturday, July 2; 30 placed in running water and 30 placed, as usual, on laboratory tables.

1st night, none spawned in either case; one male was shedding spermatozoa at 7.30 A.M. Sunday morning.

2d night, of those placed in running water one spawned one fourth maximum; of those placed on laboratory table two spawned lightly; several males shed spermatozoa in quiet water (abundant from 8 to 11 P.M.); very little in running water.

3d night, those placed on laboratory table spawned about 25 eggs; those placed in running water spawned 12 eggs; one specimen spawned in each case; males shed spermatozoa in considerable quantity in quiet water.

Remark.—Very light spawning from this group.

* New moon, July 5.

IV.

Experiment 4, July 5–7.

40 Chitons collected July 5. Placed in running water promptly, but not set for experiment night of July 5.

2d night, set in two lots, one in running water and one on laboratory table in quiet water; twenty good specimens each; of those on laboratory table one spawned 100 eggs more or less; of those in running water two spawned not to exceed 100 eggs each, between 9–11 P.M.

3d night, set as above; none spawned during the night, males nor females.

Remark.—Almost no spawning from this group.

V.

Experiment 5, July 7–9.

80 Chitons collected in afternoon, July 7. Divided into two lots, 40 each. Medium size to large specimens. Good lot. One group placed in running water and one in quiet water on laboratory table, as usual, about 7 P.M.

1st night, none spawned in either dish, males nor females.

2d night, eggs laid by two females in quiet water, about 100 eggs each or one tenth maximum; spawned at 11 P.M.; none laid by those in running water; one male shed great cloud of spermatozoa in quiet water.

3d night, no eggs and no spermatozoa obtained in either dish.

Remark.—Spawning at this time and for several days past practically suppressed.

* First Quarter, July 11.

VI.

Experiment 6, July 12–14.

60 Chitons collected in afternoon, Tuesday, July 12, and kept in running water at supply department night July 12.

2d night, July 13, experiment set in two dishes as usual; of those in running water none spawned; of those in quiet water on laboratory table three males shed spermatozoa freely before 8 P.M., and others during the night; one female spawned lightly one fourth the maximum amount about 10 P.M.; about thirty-five additional eggs were spawned by one female during the night.

3d night, at 10.30 P.M. a few males shed spermatozoa abundantly; one female spawned and only 40–50 eggs in quiet water; one female in running water spawned about 30 eggs during the night.

Remark.—Spawning practically suppressed.

VII.

Experiment 7, July 14–16.

90 Chitons collected afternoon of Thursday, July 14. Placed about 6 P.M. in three dishes. One left in running water. Two placed in quiet water on laboratory table.

1st night, spermatozoa in small amount shed by three males before 10 P.M. in quiet water; no eggs in either dish up to 11 P.M.; about 40 eggs obtained from one female in quiet water during the night.

2d night, experiment set as usual 6 P.M.; those in quiet water began to shed spermatozoa about 7.30 P.M. (one male); between 7–10 P.M. three females spawned in quantity two thirds maximum in one dish; three others spawned in quantity in another dish (about 30 chitons per dish); no eggs obtained from dish in running water.

3d night, no eggs obtained; experiment not set until 10 P.M.

Remark.—Spawning distinctly revived. For ten days preceding no eggs obtainable for class work. Was unable to get eggs for embryology class during first half of July. Spawning revived three days before full moon.

VIII.

Experiment 8, July 16–19.

1st night, about 50 chitons placed in crystallizing dish on laboratory table at 10 P.M. (too late). (Collected during afternoon of July 16.) No eggs first night.

2d night, five specimens spawned rather lightly one fourth maximum; one mass of considerable size, three fourths maximum or more; eggs in addition scattered all over the bottom of the dish.

3d night, two or three females spawned lightly before 10 P.M.; very few eggs; one fair-sized group.

4th night, no eggs.

Remark.—Spawning by this group was not particularly heavy. More eggs were spawned by previous group July 15. Not a great difference and not inconsiderable.

* Full moon, July 19.

IX.

Experiment 9, July 20–22.

40 specimens collected July 20. Placed on laboratory table at 6 P.M. Three females spawned lightly before 10 P.M. About 50 eggs each. None after 10 P.M.

2d night, placed on laboratory table at 7 P.M.; three or four spawned one third to one half maximum or more before 10.30 P.M., mostly before 9 P.M.; spermatozoa abundant; none after 11 P.M.

3d night, four females spawned about twenty eggs each; practically none at all.

Remark.—Only fair spawning activity. Plenty of eggs for class, however, and approximately equal to the two preceding experiments, 7 and 8. Spermatozoa were abundant.

X.

Experiment 10, July 25–26.

70 specimens collected Monday, July 25. Mostly half or two thirds grown, but sexually mature.

1st night, five spawned, one half to two thirds maximum.

2d night, six or seven spawned; four of them one half to two thirds maximum; impression of fair or good spawning activity; good quantity as spawning goes this year; perhaps as heavy as the best this season; comparable to that of the last week in June, but probably not quite equal to it.

* Last Quarter, July 27.

XI.

Experiment 11, July 27–29.

75 Chitons collected Wednesday, July 27. Placed in two dishes upon laboratory table. None spawned 1st night, except a very few eggs from two or three individuals. Negligible.

2d night, six specimens spawned about one third to one half maximum; all spawned before 10 P.M.; mostly between 8–10 P.M. (There were only 45 specimens used this 2d night. Several had been lost.)

3d night, no eggs.

Remark.—Good spawning activity.

XII.

Experiment 12, August 1–3.

100 Chitons collected Monday, August 1.

1st night, placed in three dishes on laboratory table; three females spawned very lightly before 10 P.M. about one fifth maximum or less; none after 10 P.M.

2d night, two dishes placed on table; one left in running water; about 75 specimens in all, some having been lost; four females spawned about 25 eggs each by 10 P.M.

3d night, no eggs.

Remark.—Spawning greatly reduced and all but suppressed. Spawning this year much lighter than last year.

XIII.

Experiment 13, August 5–6.

40 Chitons collected Friday, August 5.

1st night, placed in two dishes at 7 P.M.; no eggs or only one half dozen eggs from one female; one male shed spermatozoa before 10 P.M.

2d night, four females spawned about two thirds maximum and a fifth one half maximum; impression of fair spawning activity; good for this year; all done before 11 P.M.

3d night, about 25 eggs from each of three females; practically none; done before 11 P.M.

* New moon, August 3.

XIV.

Experiment 14, August 9–10.

60 Chitons collected Tuesday, August 9, but not brought to laboratory until next day.

2d night, two females spawned about one fourth maximum.

XV.

Experiment 15, August 10–12.

25 Chitons collected August 10—Wednesday.

1st night, none spawned.

2d night, two spawned one fourth maximum; *very light.*
3d night, one female spawned about one sixth maximum.

Remark.—Spawning very light. Almost none.

* First Quarter, August 10.

XVI.

Experiment 16, August 12–14.

45 Chitons collected Friday, August 12. Placed in two dishes.
1st night, none spawned.
2d night, three females spawned (two one fifth maximum; one one half maximum; two in one dish and one in the other); a fourth specimen spawned 50–100 eggs after 10 P.M.
3d night, no eggs.

Remark.—Impression of light spawning activity. Spawning all but suppressed during past week. Quantity of eggs not sufficient for embryological studies.

XVII.

Experiment 17, August 15–17.

100 Chitons collected Monday afternoon. Arrived 5 P.M. Put in dishes 7.30 P.M. Four dishes.
1st night, no eggs.
2d night, abundance of spermatozoa shed; water clouded; also quantities of eggs from three to five females in each dish one third to two thirds maximum.
3d night, August 17, two females spawned one fourth maximum; spermatozoa very abundant, but very few eggs.

Remark.—Very distinct revival of spawning activity; most abundant this year.

* Full moon, August 18.

XVIII.

Experiment 18, August 18–20.

50 large Chitons collected August 18.
1st night, animals remained in aquarium in laboratory (not discovered).

2d night, set in two dishes on laboratory tables; one female of medium size spawned maximum quantity (approximately 3,000 eggs); two others spawned one sixth to one fourth maximum; a fourth laid a few eggs, all before 10 P.M.; an enormous quantity of spermatozoa shed; white cloudy water.

3d night, August 20, no eggs; small amount of spermatozoa.

Remark.—Not many females spawned, but the greatest mass of eggs obtained during this year came from one female of this group. Spermatozoa were particularly abundant. An abundance of spermatozoa has been noted at each full moon, water becoming so turbid that one could not see eggs on the bottom of the shallow dish.

XIX.

Experiment 19, August 22–23.

55 Chitons collected Monday, Aug. 22.

1st night, no eggs.

2d night, only about 50 eggs from a single female; no visible spermatozoa.

Remark.—Practically no spawning.

XX.

Experiment 20, August 24–27.

60 specimens collected Wednesday, August 24.

1st night, no eggs.

2d night, one female spawned two thirds maximum between 8–10 P.M.; one male shed spermatozoa; two other females shed eggs before 11.30 P.M., about 300 and 100, respectively; a fourth female spawned during the night after 11.30 P.M. one half to two thirds maximum.

3d night, two spawned one third maximum before 10 P.M.

Remark.—Fair spawning activity.

* Last Quarter, August 26.

XXI.

Experiment 21, August 26–28.

Chitons collected from both Nobska Light and Vineyard Haven, August 26. About 100 specimens.

1st night, no eggs.

2d night, no eggs up to 10 P.M.; one female spawned 20 eggs after 10 P.M.

3d night, one female spawned 100 eggs.

Remark.—Practically no spawning. Many specimens used. Season rather late. Spawning sporadic.

XXII.

Experiment 22, August 29–30.

60 Chitons collected August 29.

1st night, no eggs.

2d night, six females spawned very lightly one fifth to one tenth maximum; only a few eggs each; several males shed spermatozoa.

Remark.—Represents a distinct increase over preceding nights. None after 10.30 P.M. Spawning per individual pretty light, however.

XXIII.

Experiment 23, August 31–September 1.

120 Chitons collected August 31. Placed in three dishes.

1st night, no eggs.

2d night, nine females spawned; three one half maximum, others one fourth to one fifth maximum; very considerable spawning and much spermatozoa in one dish; two dishes with little spermatozoa.

3d night, no eggs.

Remark.—Good spawning activity for time of season.

* New moon, September 1.

XXIV.

Experiment 24, September 6–7.

85 Chitons, collected Tuesday, September 6. Placed in three dishes.

1st night, no eggs.

2d night, two females spawned in one dish two thirds maximum; one spawned in another dish one fourth maximum, 8–10.30 P.M.; third dish, no eggs.

Remark.—Experiments for the season closed September 7. For some days there had been indications that the spawning season was nearing its end. Impression of fair spawning activity, although from only a few individuals. The experiments show that heavy spawning begins within a few days of full moon and continues to third quarter, or a few days after third quarter. The lightest spawning occurs between new moon and full moon, especially pronounced around first quarter.

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NOTES ON THE LOCOMOTION OF THE NUDIBRANCHIATE MOLLUSK DENDRONOTUS GIGANTEUS O'DONOGHUE.*

H. P. KJERSCHOW AGERSBORG.

Many works have already appeared on molluscan locomotion. Thus Simroth ('79) called attention to the relation between the pedal nerve, the musculature of the foot, and the "locomotorischen Wellen" in *Limax*. Fleischmann ('85) observes: "Kontraktionswellen über die ganze Oberfläche des Fusses in *Anodonta,* hinten beginnend und nach vorn strichend." Jordan ('01), and again in 1905, records a similar feature for *Aplysia limacina*. Bohn ('02) finds that progressive movements of *Helix pomatia* L. are accomplished by undulations in the form of waves of the pedal musculature which rests against a solid or support, and that these rhythmic pedal waves are partly independent of mechanical excitations. Künkel ('03) finds that the "Wellenspiel" increases when he touches a crawling *Limax tenellus* (or *L. agrestris, L. arborum*). Carlson ('05) substantiates the findings of Jordan ('01) by describing for *Helix dupetithonarsi* a succession of large waves which pass from the head to the tail, an extraordinary mode of progression employed when the animal is in a hurry. Biedermann ('05) refers to "Querbänder" that follow one another in parallel lines on the under side of the foot of *Helix pomatia,* when this species crawls on a glass plate. Von Uexküll ('09) confirms the findings of Jordan for *Aplysia,* viz., that waves are advancing, when the foot is lifted from the ground, from the anterior to the posterior, the waves advancing in the opposite direction in *Limax* and *Helix*.

A more complete analysis of pedal locomotion among mollusks and other invertebrates has been accomplished by the brilliant works of Parker ('11, '14, '17, '17*a,* '21) and his pupil, Olmsted ('17). Parker ('11) recognizes two main sets of locomotion means for ordinary gastropods: (1) arhythmic (*Ilyanssa obsoleta*

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* Contribution from the Zoölogical Laboratory of the University of Illinois, No. 200.

Say) locomotion is accomplished without pedal waves; (2) with pedal waves, or rhythmic locomotion (*Dolabrifera virens* Verrill, *Tectarius nodulosus* Gmel., *Nerita tessellata* Gmel., *Chiton tuberculatus* Linnæus, etc.). "In rhythmic locomotion the waves may run from the posterior to the anterior, that is, *direct;* or the reverse, that is, *retrograde*. The foot may exhibit one, *monotaxic;* two, *ditaxic;* or four, *tetrataxic,* series of waves. In the ditaxic foot the waves may be alternate or opposite. . . . Locomotion is the cumulative result of local forward motion on the part of one section of the foot after another till the whole foot has been moved." The essential act of pedal locomotion of all creeping gastropods may be exhibited by the retrograde wave movement on the foot of *Chiton tuberculatus,* in which, according to Parker ('14), the locomotor waves run from anterior to posterior. In this type, "The waves extend the whole width of the foot and are from five to six mm. in antero-posterior extent. They represent an area of the foot temporarily lifted from the substrate. . . . Excepting in the region of the wave, the foot is firmly attached to the substrate; hence at any moment from nine tenths to four fifths of the foot is fixed and the remainder free." Pedal locomotion of Actinians (*Metridium marginatum* Milne-Edw., *Sagartia luciæ* Verrill, *Condylactis passiflora* Duch. and Mich., and *Actinia bermudensis* Verrill) is interestingly accomplished by a wave-like movement which progresses over the pedal disc in the direction of locomotion (Parker, '17). "In the actinian locomotor wave each point on the pedal disc is successively raised from the substratum, moved forward, and put down." Writing on the locomotion of the sea-hare, *Aplysia californica* Cooper, this same author ('17*a*) says: "Pedal locomotion in *Aplysia* is due to monotaxic retrograde waves which lift the foot locally and temporarily from the substrate, making it thus to move forward with freedom, while the rest of the foot for the time being holds the snail in place by many small areas of local suction. The portion of the foot that moves forward is the elevated region," pp. 143 and 144. Locomotion in the holothurian *Stichopus panimensis* Clark is effected by direct monotaxic waves (Parker, '21). That is, "Creeping is accomplished in part by a muscular wave that originates at the posterior end of the animal and sweeps over it to the anterior." It is interesting to note that locomotion in this echinoderm is in essentials of that type of gastropod locomotion which has been designated the direct monotaxic type.

Olmsted ('17) finds in the huge slug *Veronicella schivelyoe* Pilsb. there are always about eleven pedal waves which pass from the posterior to the anterior and extend the full width of the foot; this is direct monotaxic locomotion. In *Onchidium floridanum* only one or two waves are shown at a given time. In *Eulota simularis* Fer. the average number of waves are 9 à 10. *Helcinia convexa* Pfr., *Tethys dactylonula* Rang, and *Fissurella nodosa* Born show retrograde monotaxic locomotion. *Tectarius misricatus* L. exhibits retrograde alternate ditaxic locomotion. *Tritonidea tincta* and *Columbella mercatoria* L. are retrograde tetrataxic in their locomotion. *Cypræa exanthema* L. shows long and short lateral waves which move either to the right or left; complete diagonal waves which move to the right or left, and retrograde waves from the anterior edge to the center of the foot and extends to the posterior. Finally, *Marginella arena* Val., *Haminea antillarum* Orb., and *Bulla occidentalis* A. Ads. move by ciliary action alone. Retrograde pedal waves, according to Crozier ('19), are the means of locomotion in *Ischnochiton purpurascens* Ad., *Acanthochites spiculosus* Reeve, and *Tonicia* sp.; the first-named one also exhibits a "gallop" like that of *Helix,* which is independent of the pedal waves.

The remarkable nudibranch *Dendronotus giganteus* has recently been described (1921) from the Vancouver Island region by Dr. Chas. H. O'Donoghue. This author records four species of this genus: *D. arborescence* Müller, *D. dalli* Bergh, *D. giganteus* O'Donoghue, and *D. rufus* O'Donoghue, from this region. The largest of these species measured: 50 mm. long by 15 mm. high by 15 mm. broad; 31 mm. by 9 mm. by 6 mm.; 140 mm. by 43 mm. by 33 mm.; and 14 mm. by 4.5 mm. by 4 mm., respectively. According to this author, a preserved specimen of *D. giganteus* in the laboratory obtained by Professor C. M. Fraser, 1913, measured 210 mm. long by 84 mm. high by 55 mm. wide. Allowing for approximately the same amount of shrinkage, according to O'Donoghue, the specimen when alive must have reached the size of 260 mm. long by 100 mm. high and 65 mm. broad. This is indeed a gigantic size for a nudibranch! It is matter of common knowledge, however, that the fauna of the North American Pacific coast attain not only a large size, but are in fact very abundant and diversified. (*Vide:* Kjerschow Agersborg, 1920.)

Large specimens of this genus also occur in the vicinity of Puget Sound Biological Station, Friday Harbor, Washington. Thus during the summer of 1913 a collecting party from the station found on the shore of Shaw Island (not very far from Canoe Island) a large specimen which must have measured about 200 mm. in length. To the writer's knowledge no one identified the specimen at that time. It was conveniently classed *D. arborescence.*

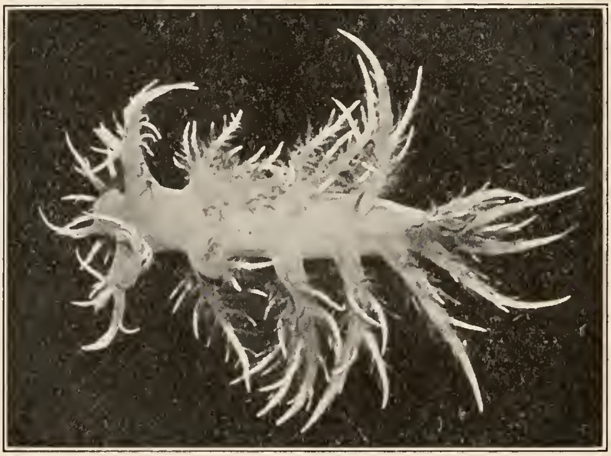

Fig. 1. Photograph of *Dendronotus giganteus* O'Donoghue, dorsal view.

During the summer of 1921 I again visited the Puget Sound Biological Station. On July 20 I found a very fine specimen of *Dendronotus* between the logs of the floating dock of the station (Figs. 1–4). Upon examination it was found to fit perfectly to the description of O'Donoghue for *D. giganteus.* It measured 140 mm. long by 60 mm. high by 40 mm. broad. The foot was 90 mm. long and 40 mm. wide at its widest part. This specimen was kept alive in the laboratory for three weeks. During this time it did not feed, as far as I know, on anything. I tried to feed it on crustaceans, echinoderm gonads, cœlenterate tentacles, green algæ, etc., but it refused all these except once, in which case it opened its large fleshy lips in response to the touch of cœlenterate tentacles, took them within its mouth, only to be ejected a little later. When the animal was first found, and for several days afterwards, its stomach was filled with air, which aided it in floating. It was able, however, to submerge itself while in this condition. When the air bubbles contained in the stomach after a few days had disappeared, a little by little, the animal was able to float nevertheless.

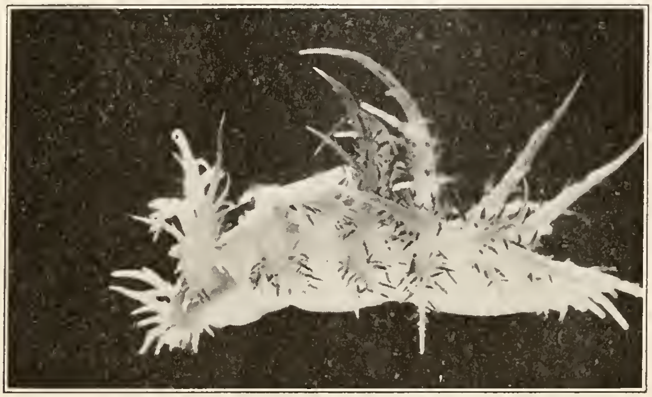

FIG. 2. Photograph of *Dendronotus giganteus* O'Donoghue, lateral view.

In conjunction with the study of qualitative chemical and physical stimulations in *Hermissenda opalescence* Cooper, I also studied *D. giganteus* (*vide* Kjerschow Agersborg, 1922*a*; for a full description of the species: O'Donoghue, 1921), and noticed incidentally its remarkable mode of locomotion. During the first few days in the laboratory it was very active, and it was quite difficult to make proper observations on its response to stimuli; on the other hand, it offered the opportunity to study its mode of locomotion which is appended.

Dendronotus giganteus has two distinct modes of locomotion. The one, and the most commonly used, is that of swimming; the other is creeping. The swimming movements are effected by a regular twisting of the body in an undulatory manner, beginning at the anterior end and passing gradually to the posterior. These movements are alternated from side to side. The lashing of the anterior part of the body from side to side is relatively powerful and a much more rapid progressive movement is effected over that attained by creeping. The undulatory movements employed in swimming start with the head being bent downward and sideways, forming a wave-like twist in the side of the body-wall like that in a blade of a propeller. This wave passes gradually toward the posterior end (Figs. 3–4), and disappears when the animal makes the next stroke to the opposite side. When the animal makes a stroke to the right the posterior two thirds is bent so as to form an angle of 45° with the anterior one third. But the posterior part of the body also rotates about 45° from the vertical plane, so that the left side with the foot forms a large wave which sweeps posteriorly, while the anterior part of the body, in front of the wave, is kept vertically. When the animal makes a stroke to the left, the same phenomenon is repeated on the right side. An animal may cover a distance of 30 cm. in a few seconds, making side-strokes of about 45 a minute.

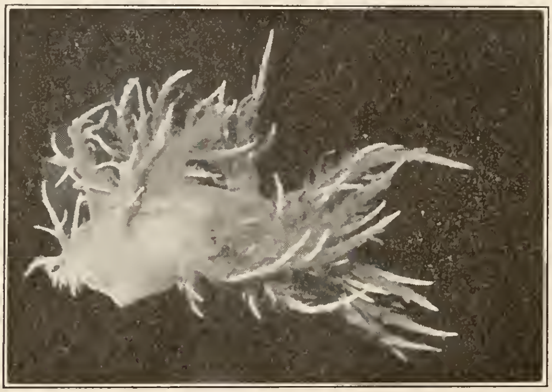

FIG. 3. Photograph of *Dendronotus giganteus* O'Donoghue in the act of swimming; the head has returned from its bend to the right, and the twist-like wave is shown in the side passing toward the posterior end.

The creeping method of locomotion was seldom employed. If the animal was forced to the bottom of the dish, it would attach itself to the substratum and commence gliding along imperceptibly. Close observation brought to light, however, a succession of undulations which passed from the posterior end of the foot to the anterior. The animal adhered to the substratum by sucking to the extent that it did not pass out of the dish when the water was poured out of it. In creeping, local undulations are set up in the form of series of areas adhering to the substratum, and intervened by non-adhering parts which travel gradually toward the anterior end. The method of creeping may be classed as direct rhythmic locomotion, to use the terminology of previous writers on this subject, although it is not possible to classify this exactly at this time. *The method of rhythmic locomotion is accompanied in this case, I believe, by ciliary action. The foot in *Dendronotus giganteus* is highly ciliated. No slime is produced during locomotion or at any other time. The foot is also uniformly ciliated in *Melibe leonina* Gould (Kjerschow Agersborg, 1921*a,* 1922). Ordinary locomotion in *Melibe* is effected by the ciliary action of the foot. The ciliated epithelium is innervated with nerve fibers from the pedal nerve-net (Kjerschow Agersborg, 1922).

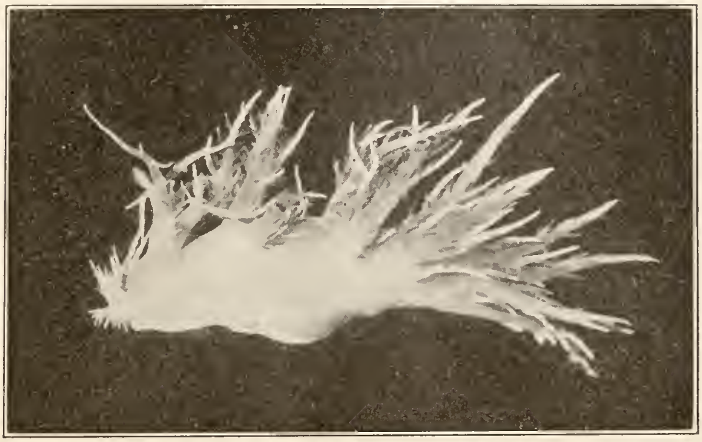

FIG. 4. Photograph of *Dendronotus giganteus* O'Donoghue showing the locomotor wave during swimming passing toward the posterior end. All the photographs are of living specimens, photographed through the water, by Dr. Myrtle E. Johnson, State Teachers College, San Diego, Calif. The photographs were taken at the Puget Sound Biological Station, July, 1921.

Swimming as a means of locomotion is common among pelagic forms which are then frequently provided with secondary organs for that purpose as in *Aplysis* Linnæus, in which the parapodia figure quite prominently. Forms closely related to the nudibranchs, such as the gymnosomatous *Pteropoda,* may be particularly exemplified in this connection. These parapodic forms are pelagic *per se* (*vide* Kjerschow Agersborg, 1922*c*). Although several nudibranchs are pelagic, this habit is secondary, notwithstanding.

Collingwood ('79) records swimming as a means of locomotion in *Scyllea pelagica,* and Garstang ('90) for *Lomanotus* Verany. Kjerschow Agersborg ('19, '21, '22, '22*b*) reports swimming as one mode of locomotion in *Melibe leonina.* But none of the forms as here mentioned are good swimmers although Garstang's statement seems to indicate that *Lomanotus* progresses through the water during swimming. I am unaware of any previous record as to the pelagic habit of *Dendronotus.* The assumption may be quite justified that *Dendronotus,* in spite of its highly developed foot, is pelagic in habit, not only because of the evidence connected with the place of discovery of the particular specimen upon which this discussion is based, but rather on the striking, highly developed method of locomotion, viz., swimming. *Melibe leonina,* although quite frequently pelagic in habit, is not such an able swimmer as *Dendronotus giganteus.* The last-named species may, therefore, be more pelagic in habit than it is commonly thought to be.

Simroth, Fleischmann, Jordan, Bohn, Künkel, Carlson, Biedermann, von Uexküll, and others have shown that during ordinary locomotion not only do the muscle fibers of the foot take part in the production of the pedal waves, but also certain muscle fibers of the body-wall. This is more readily understood when one realizes the relation of the muscle fibers of the body-wall to those in the foot, in terrestrial as well as in aquatic or marine snails. The swimming habit, of course, is not equally developed in all, which is partly due to the general shape of the body. During swimming the muscle fibers of the body-wall act preëminently; during creeping, those of the foot.

SUMMARY.

1. *Dendronotus giganteus* O'Donoghue swims by bending the anterior end of the body sideways to an angle of about 45°, pro-

ducing each time a powerful stroke by the head, and forming in the right or left side of the body-wall, according to the bend of the head, a large twisted muscular wave which passes toward the posterior. By the sweep of these waves locomotion is effected. Of all known non-parapodic nudibranchs, *Dendronotus giganteus* is the most able swimmer.

2. Creeping is effected by direct rhythmic waves augmented by ciliary action of the foot.

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NON-CRISS-CROSS INHERITANCE IN DROSOPHILA MELANOGASTER.

LILIAN V. MORGAN.

A complete reversal of the ordinary criss-cross inheritance of recessive sex-linked characters occurs in a line of *Drosophila* recently obtained. In ordinary sex-linked inheritance the recessive sex-linked characters of the mother are transmitted to the sons, while the daughters show the dominating allelomorphic characters of the father. In the present case the daughters show a recessive sex-linked character of the mother and the sons show the dominating allelomorphic character of the father. The reversal is explicable on the assumption that the two X-chromosomes of the mother are united and behave at reduction as a single body. Sections show that the eggs of these females do have two united X-chromosomes; the cytological evidence verifies the genetic deduction.

A female fly (Fig. 1, *a*), which was a somatic mosaic, showing different sex-linked characters in the anterior and posterior parts, appeared in a pair culture of which the parents had the following constitution: one X of the mother carried the differentials for eosin, cut and forked; the other X carried the differentials for forked and bar. She was therefore forked, heterozygous bar, and gray. The X of the father carried the differential for yellow body color, and he was therefore yellow.

The head and thorax of the mosaic (Fig. 1) were gray and the eyes were slightly bar, while the abdomen was yellow; the fly was entirely female, having heterozygous bar eyes, no sex-combs, and normal female genitalia. She was not virgin, but nevertheless was isolated and mated to a black male, and produced 43 daughters and 59 sons. The daughters were, without exception, all yellow and the sons were all gray, and all the offspring had wild type eyes—*i.e.,* none were bar except two sons that were also forked and will be considered later. The conclusion was at once evident that the mosaic fly had received from its father two yellow-bearing chromosomes, inseparable from one another, and that these inseparable

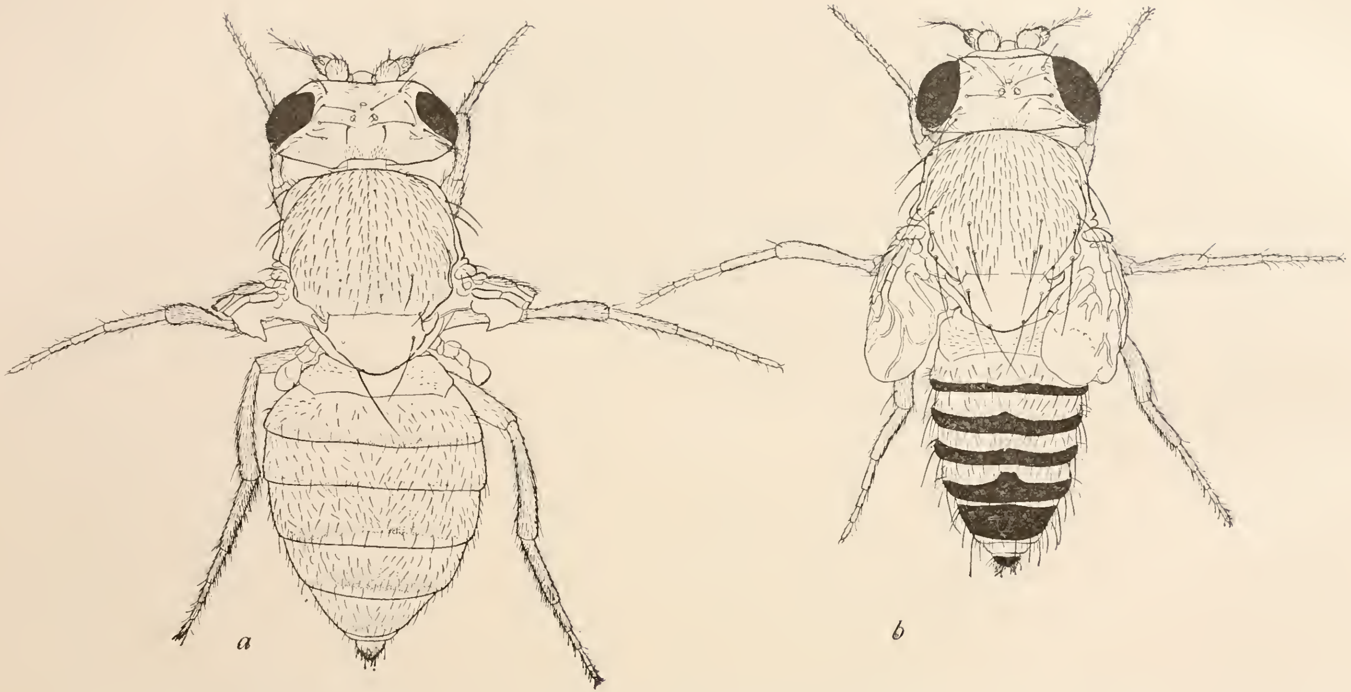


Fig. 1. *a,* Mosaic female fly (wings cut off); head, thorax (grey wings) and legs, wild type color (heterozygous for black); eyes slightly Bar; balancers and abdomen yellow (with light bands of yellow type). *b,* Triploid-X female, "grey" with abnormal wings, and flat, small abdomen.

chromosomes were transmitted together to the next generation, producing (wherever they occurred) females, because there were always two of them. No male offspring could be yellow, because no single yellow chromosome was transmitted (see Fig. 2). The sons must receive their X-chromosome from their father. The mosaic, as already stated, was not virgin; her brothers were all forked and some of them were bar, so that the two forked bar sons were in all probability offspring of a first mating and the other sons were offspring of the black male.

Three of the F_1 females were mated to three brothers, but no one of the pairs produced offspring; neither did a mass culture of F_1 males and females, nor did three F_1 males outcrossed to yellow-white females have any offspring. On the other hand, F_1 females outbred were fertile, and the sons of these were also fertile. The sterility of the F_1 males was expected if the X-chromosomes of the mosaic parent remained together, as the analysis shows, for then she should have produced two kinds of eggs (cf. Fig. 2), one kind with the double X and one kind with no X-chromosome. The XX-eggs fertilized by X-sperm would have produced XX X females (triploid-X females which would die); the XX-eggs, fertilized by Y-sperm, would have produced XXY females, the yellow daughters. The no-X or O-eggs, if fertilized by X-sperm, would have produced XO males, in appearance like the father, but sterile because lacking a Y-chromosome, and the O-eggs fertilized by Y-sperm would have produced YO males which would have died. Half the F_1 males and half the F_1 females presumably died and the sex-ratio remained 1 : 1. The actual numbers were 43 females to 59 males. The daughters were all yellow, but differed from their yellow mother in having, besides the "yellow-bearing" double chromosome, a Y-chromosome from their father; their sons therefore received from them a Y-chromosome and were fertile, and the surviving half, receiving their X-chromosome from the father, resembled him. Such patroclinous sons were produced by mating F_1 yellow daughters to wild type, to yellow white, to Xple, and to yellow broad, eosin, ruby males. The ratio of females to males in 8 cultures was 435 : 437, as would be expected again in this generation, since out of the four classes one class of females (XX X) would die, one class (XX Y) would survive, one class of males (XY) would survive, and one class (YY) die (Fig. 2). In subsequent generations the line of double yellow females has remained intact; crosses have been made to many more types of males, always producing males like the father. The double yellow females are now used when it is desired to keep a supply of a certain type of male, for, since the X-chromosomes are inseparable, any egg which receives them produces a female, and only those eggs which receive the father's X can produce males. In such a line, then, there is never any crossing over in the X-chromosomes, and any desired combination of sex-linked characters can be maintained in the male flies by mating a male having the characters to a double yellow female. This is useful in cases where females of the race are weak or sterile, or where the pure cultures of the desired characters are weak, as frequently happens with a complicated combination of characters. The double yellow females are very vigorous and afford a reliable medium upon which to rear a race of feeble males. A possibility of a break in the continuity of a pure culture, which is easily discovered and rectified, will be discussed later.

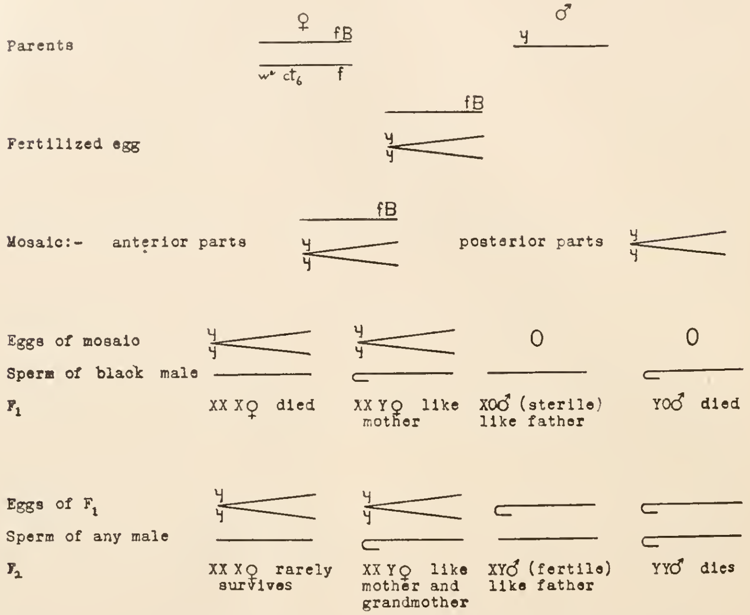
The double yellow females probably behave like other flies in regard to the inheritance of characters in the autosomes. The original mosaic was dichete and transmitted the character normally. Crossing over in the second chromosome was tested by mating double yellow females to black purple vestigial lobe males and was found to be normal. Crossing over in the third chromosome was tested by mating double yellow females to pink, spineless, kidney, sooty, rough males, and found to be normal at the left end of the chromosome, but not at the right end; the discrepancy may, however, be due to the difficulty in classifying sooty in the presence of yellow.

X-TRIPLOIDS.

After many hundreds of flies had been examined, there appeared occasionally, in cultures where the males were gray, wild type females of a more or less abnormal appearance; the eyes were somewhat rough and smaller than normal, the wings more or less imperfect and sometimes serrated, the abdomen imperfect, and the flies were weak (Fig. 1, *b*). These are characteristics of females triploid for X. It is clear that from time to time a three X female survives. She has gray wings and body color like the father, because two yellow genes are recessive to one wild type gene. In a culture where the father was bar, the X-triploids had eyes less bar than in a heterozygous female, yet showing the character bar; this is known to be the appearance of bar when carried in one chromosome in the presence of two other chromosomes, both carrying the gene for wild type. X-triploids have not been observed in all of the cultures, and the percentage of them when present varies very much. In one culture their number was for a time about equal to the number of yellow females. All of many attempts to breed them have failed.

Occasional Breaking Apart of the Double Chromosome.

No exception to the non-criss-cross inheritance was observed until the F_4 generation, when a single yellow male appeared in a line in which all the other males were wild type. The yellow chromosomes of the mother had in this case presumably broken apart and the usual type of sex-linked inheritance from mother to son had been restored. The fly bred like an ordinary yellow male. At about the same time, in each of two related cultures of double yellow females by cross-veinless, cut, forked males, there appeared a wild type female not having the characteristics of an X-triploid. Both of these flies were fertile and both bred like females having one yellow-bearing X-chromosome, and one X-chromosome bearing the characters of the males of the cultures—*i.e.*, having the differentials for cross-veinless, cut and forked. One of these females was bred to a brother and produced females of two classes (wild type and cross-veinless, cut, forked) and yellow sons, and cross-veinless, cut, forked sons and the cross-over classes to be expected. The other wild type female was bred to a 7ple male and also produced yellow sons and cross-veinless, cut, forked sons, and females and cross-overs of the classes to be expected. These two females, like the yellow male of the other culture, had received from the mother a single yellow chromosome, broken apart from its mate. Since these flies occurred there have appeared in cultures of double yellow females six other yellow males and two wild type, not X-triploid, females. All the males have been bred and all but one have produced offspring of the expected classes. Another female of the same kind undoubtedly occurred in a mass culture of double yellow females, for there were found in the culture some yellow males and cross-over males and females of classes to be expected if a female heterozygous for yellow had been present in the culture.



Parents
♀
fB
w^e ct_6 f
♂
y
Fertilized egg
fB
y
y
Mosaic:- anterior parts
fB
y
y
posterior parts
y
y
Eggs of mosaic
Sperm of black male
F1
XX X♀ died
XX Y♀ like mother
XO♂ (sterile) like father
YO♂ died
O
O
Eggs of F1
Sperm of any male
F2
XX X♀ rarely survives
XX Y♀ like mother and grandmother
XY♂ (fertile) like father
YY♂ dies

FIG. 2. Diagram showing parentage and constitution of the mosaic and of the line of non-criss-cross inheritance. The components of the double chromosome are arbitrarily represented as attached at "left" end.

The question as to whether the break occurs in the place of the original attachment of the chromosomes has not been fully tested, but so far there is no evidence of duplication or deficiency at either end of the detached chromosome.

CYTOLOGY.

The genetic behavior of the line of flies having the two inseparable sex-chromosomes is in entire accord with the condition of the chromosomes as seen in cytological preparations of yellow females and of their rare wild type XX X sisters. Both of these classes of females receive from the mother not a single X, but two

X's attached to one another; the yellow female should have, in addition, a Y-chromosome from her father (Fig. 2, last line), the wild type female an additional X-chromosome from her father. Such chromosomes are, in fact, found in the maturation stages of the eggs of these females (Fig. 3, *b*, *c*, *d* and *e*, *f*, *g*, *h*). In both

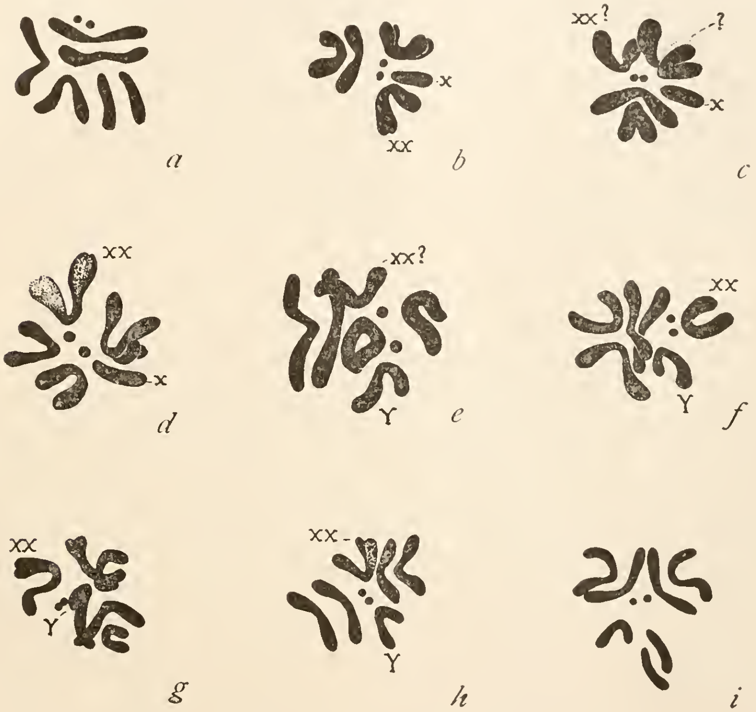


FIG. 3. Oögonial plates of: *a*, wild type female (after Bridges); *b*, *c*, *d*, triploid-X grey females (described in the text); *e*, *f*, *g*, *h*, double yellow females (described in text); *i*, non-disjunctional female of another origin (after Bridges), having three sex-chromosomes, shown for comparison. In all the figures are three pairs of autosomes. In the wild female (*a*) are two rod-shaped X-chromosomes (one from the father and one from the mother). In the triploid-X (*b*, *c*, *d*) and the double yellow (*e*, *f*, *g*, *h*) females is a single V-shaped body, the two attached X-chromosomes from the mother (sometimes with difficulty distinguishable from an autosome). In addition to these, there is: in the triploid-X females (*b*, *c*, *d*), a *rod-shaped* typical X-chromosome (derived from the father and accounting for the grey color of the fly); in the yellow females (*e*, *f*, *g*, *h*), the typical Y-chromosome (from the father). In the non-disjunctional female previously described by Bridges (*i*), the three sex-chromosomes are a Y-chromosome and two X-chromosomes not attached by their ends.

types there are the usual three pairs of autosomes, but the sex-chromosomes differ from the usual condition in that there is present in both types a U- or blunt V-shaped chromosome in place of a rod-shaped X-chromosome (Fig. 3, cf. *a* with *b, c, d* and *e, f, g, h*); this body is undoubtedly the united X's derived from the original mosaic. Besides the united X's from the mother, there is in the yellow female the typical hooked Y-chromosome, the sex-chromosome from the father (Fig. 3, *e, f, g, h*), and in the wild type female the chromosome from the father is the typical rod-shaped X (Fig. 3, *b, c, d*), whose presence gives her the wild type body color of her father. Thus the cytological evidence brings another confirmation of the chromosome theory of heredity.

Origin of the Mosaic.

The origin of the mosaic fly can be explained if at some division in the spermatogenesis of the father (perhaps at the equation division) the two halves of the X-chromosome failed to become completely detached, but remained fastened together at one of their ends, producing the V-shaped chromosome or double chromosome found in the germ cells of the female descendants. The sperm containing this chromosome must have fertilized an egg containing an X-chromosome with the differentials for forked and bar (this is known to have been the constitution of half of the eggs of the mother). An X-triploid fly was thus produced (cf. Fig. 2); the triploid condition remained, however, in only the anterior part of the fly, where the body color was wild type and the eyes were slightly bar. These characters, as stated, are as expected in an X-triploid, where two differentials for yellow are present with one for wild type, and one differential for bar with two for wild type. At an early stage the chromosome carrying forked and bar was eliminated, leaving part of the fly (the abdomen) with only two X-chromosomes, the attached yellow-bearing chromosomes (Fig. 2). The germ cells of the individual were derived from this part, and at the reduction division of the eggs there must have been extruded into the polar body (or remained in the egg) either the two attached X-chromosomes or no chromosome.

Dec. 1921.

Vol. XLII. *June, 1922.* *No. 6.*

BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY.

TWENTY-FOURTH REPORT, FOR THE YEAR 1921, THIRTY-FOURTH YEAR.

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I. TRUSTEES, AUGUST 9, 1921.

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William Patten, Dartmouth College.
Jacob Reighard, University of Michigan.
W. B. Scott, Princeton University.

* Deceased.

II. ACT OF INCORPORATION.

No. 3170.

COMMONWEALTH OF MASSACHUSETTS.

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT. HENRY B. PIERCE,

[SEAL.] *Secretary of the Commonwealth.*

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY.

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio,* members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio.*

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT.[1]

Harvey S. Chase & Company,
Certified Public Accountants, 84 State Street, Boston.

January 21, 1922

Mr. D. Blakely Hoar,
161 Devonshire Street,
Boston.

Dear Sir: We have completed our audit of the accounts of the Marine Biological Laboratory for the year ended December 31, 1921, as kept both at your office in Boston and at Woods Hole and report thereon in the accompanying exhibits and schedules:

Exhibit A—Balance Sheet as of December 31, 1921.
Exhibit B—Income and Expense for the Year ended December 31, 1921.
Schedule I—Investments (Book Values).
Schedule II—Cash Receipts and Disbursements on Account of Funds.
Schedule III—Land, Buildings, and Equipment.
Schedule IV—Supply Department Income and Expense Account for the Year ended December 31, 1921.

We certify that, subject to the comments herewith, the balance sheet and income and expense statement shown in Exhibits A and

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[1] Only a part of the audit is included in the Treasurer's report. The complete audit is on file at the Laboratory and may be examined by any member.

B are in accordance with the books and correct to the best of our knowledge and belief.

Very respectfully,

(Signed) Harvey L. Chase & Company,
Certified Public Accountants.

Marine Biological Laboratory Balance Sheet, December 31, 1921.

Assets.

Cash:			
In bank		$1,548.37	
Petty cash fund		200.00	$1,748.37
Notes receivable (secured)			1,000.00
Accounts receivable			9,687.22
Inventories:			
Supply department		20,152.90	
Biological Bulletin		3,535.95	23,688.85
Investments:			
Securities (Schedule I.)	$11,694.87		
Less loan	1,500.00	10,194.57	
Cash (Schedule II.)		809.87	11,004.44
Stock in General Biological Supply House, Inc.			12,700.00
Gansett property account		20,754.95	
Less mortgage		11,582.01	9,172.94
Educational Plant (Schedule III.)			
Land		95,856.14	
Buildings		207,855.06	
Equipment		98,465.02	
		400,176.22	
Less reserve for depreciation		42,070.17	358,106.05
Deferred charges:			
Prepaid insurance			2,786.43
Items in suspense			336.98
			$430,251.28

Liabilities.

Accounts payable		$2,863.84
Notes payable:		
Falmouth National Bank		10,000.00
Accrued charges (estimated)		1,000.00
Items in suspense:		
Friendship Fund donation for 1922 expenses	$5,000.00	
Amount received from Marine Biological Laboratory Club for repairs to club house	500.00	
Other items	105.87	5,605.87
Trust funds		11,004.44
		$30,474.15

Balancing Account:			
Balance, January 1, 1921		$385,582.48	
Add:			
Special Donations received during year for completing third floor of New Laboratory Building:			
Nela Research Laboratory	$3,000.00		
Eli Lilly Co.	3,000.00		
Friendship Fund, Inc.	1,500.00	7.500.00	
Balance of Income Account for Year (Exhibit B)		3.994.65	
Special Donations received in 1919 and 1920 towards purchase of stock of General Biological Supply House		2,700.00	399,777.13
			$430,251.28

Marine Biological Laboratory, Income and Expenses for Year Ended December 31, 1921.

	Expenses	Income	Loss	Gain
Administration expenses	$9,657.82		9,657.82	
Bar Neck Property expense	340.82		340.82	
Biological Bulletin and annual dues	4,186.35	3.537.15	649.20	
Biological Bulletin, adjustment of expenses for 1920		347.58		347.58
Carpenter department	1,044.09	7.58	1.036.51	
Chemical department	1,763.77		1,763.77	
Dormitories	2,609.21	2.467.62	141.59	
Instruction	6,915.60	8,882.50		1.966.90
Janitor's house expenses	7.68		7.68	
Interest on notes payable	570.00		570.00	
Library department	1,979.12		1,979.12	
Maintenance, buildings and grounds	6,919.63		6,919.63	
Mess	23.264.52	24.556.41		1,291.89
New laboratory	3,994.07		3,994.07	
Newman Cottage	73.90	150.00		76.10
Pumping station	514.83		514.83	
Research department	2,605.57	6,325.00		3,719.43
Sundry expense and income	1,241.89	9,243.76		8,001.87
Supply department (See Schedule IV.)	41,561.10	42,774.78		1,213.68
Truck	720.43		720.43	
Total current expenses	109.970.40		28,295.47	
Total current income	98,292.38	98,292.38	16,617.45	16.617.45
Excess of expenses	11,678.02		11,678.02	

Reserve for depreciation		7,270.69
Bad accounts written off		204.64
		19,153.35
Donations for Expenses:		
Friendship Fund	$20,000.00	
Frances C. Lillie	3,000.00	
Other	148.00	23,148.00
Balance to balancing account		$3,994.65

Marine Biological Laboratory Investments, December 31, 1921.

Reserve Fund.

Cash on hand	$460.06	
*$3,000.00 American Telephone & Telegraph Company, 4's	2,921.25	
500.00 Western Telephone & Telegraph Company, 5's	496.88	
*6 shares American Smelting & Refining Company, Preferred	732.00	
8 shares General Electric Company	907.25	
14 shares United Shoe Machinery Corporation, Preferred	393.75	
5 shares Massachusetts Gas Companies, Preferred	444.63	
	6,355.82	
Items marked * are held as collateral on loan of	1,500.00	$4,855.82

Library Fund.

$300.00 U. S. Liberty Loan, 4 1/4's	300.00	
4/5 of $1,000.00 American Telephone & Telegraph Company, 4's	779.00	
3 shares American Telephone & Telegraph Company	372.90	
3 shares General Electric Company	346.80	
5 shares United Shoe Machinery Corporation, Preferred	140.63	
3 shares Massachusetts Gas Companies, Preferred	269.38	
1 share American Smelting & Refining Company, Preferred	122.00	
	2,330.71	
Overdraft of cash	261.84	2,068.87

Lucretia Crocker Fund.

Cash on hand	611.65	
$300.00 U. S. Liberty Loan, 1st Convertible 4 1/4's	300.00	
1/5 of $1,000.00 American Telephone & Telegraph Company, 4's	194.75	
18 shares Vermont & Massachusetts Railroad Company	2,416.50	
3 shares General Electric Company	349.55	
1 share West End Street Railway Company	83.00	
1 share American Telephone & Telegraph Company	124.30	4,079.75
Total—Exhibit A		$11,004.44

V. REPORT OF THE LIBRARIAN.

The Library has enjoyed a steady growth since the previous report, the total number of books added to the Library being 929. Of these 78 volumes were received by purchase, 293 by binding of periodicals, 105 as gifts, and 26 as permanent loans from the American Museum of Natural History. To these should be added 427 reprints, mostly in pamphlet form. During the year the number of periodicals received has been 260 titles, of which 97 are received by subscription, 52 by exchange for the BIOLOGICAL BULLETIN, 99 are gifts, one is received by advertising, and 11 periodicals are sent to us from the duplicates of the American Museum of Natural History as loan. At the end of the year the total contents of the Library is 10,663 volumes and 8,959 pamphlets.

The Library has been fortunate in receiving a number of valuable gifts of books and of money. We may mention especially a gift of $50 from Miss Anna E. Lagemann, of $88 from Dr. Frank R. Lillie for the purchase of certain sets of journals, and $10 from Dr. Alfred G. Mayor for subscriptions to periodicals. Of books, we have received from the authors—

"The Nature of Animal Light," E. Newton Harvey.
"The Grand Strategy of Evolution," William Patten.
"Physiological Chemistry," 3d edition, Albert P. Matthews.
"Human Behavior," Stewart Paton.

From Messrs. Squibbs we have received their "Materia Medica," and from Messrs. Merck & Co., Benjamin L. Murray's "Standard Tests for Reagent Chemicals, 1920." An especially interesting gift is a volume of "Ramblers of a Naturalist, 1857," by de-Quatrefages. This book is given by Mr. William Beer, Librarian of the Howard Memorial Library of New Orleans. Mr. Beer visited Wood's Hole in the summer of 1920, was much interested in our Library, and has sent this book in recognition of the friendships then established. Dr. H. H. Donaldson has very kindly given us a copy of Cattell's "American Men of Science," edition 3, and from the Rev. Mr. Tingley we have received a copy of Porter, "The Human Intellect," and Barrett, "Threshold of the Unseen." The Librarian also wishes to express thanks for friendly suggestions received from various members of the laboratory, especially Dr. Pond, Dr. Osterhout, and Dr. Franklin.

During the winter preceding the opening of the laboratory the Assistant Librarian rearranged the books in the Library, placing them in three series. Ordinary books are arranged at the west end of the Library and placed in order according to the Dewey decimal classification, the classification being somewhat minute with a view to future expansion. The journals are arranged in the next section of the Library and classified into a few broad classes. Under each class the journals are arranged alphabetically by title. The reprints are massed at the east end of the Library and are all arranged alphabetically. We have been able to complete ten important sets of journals by purchase, exchange, or gifts. Among the important ones that have been completed are—

Morphologisches Jahrbuch,
Quarterly Journal of Microscopic Science,
The New Phytologist,
La Cellule,
Proceedings of the Boston Society of Natural History.

The dictionary catalogue, which was started last year, now contains 8,637 cards. The use of the Library has increased considerably, the circulation for the year 1921 being 1,200 items. The new forms which have been devised for keeping records have proved very satisfactory and useful.

The needs of the Library are numerous and various. We may mention especially (1) reprints, of which none have been received recently from members of the laboratory. Authors are requested to look over the reprints in the Library when visiting Wood's Hole and to send us what are lacking; (2) larger appropriations are needed for the purchase of books much in demand; (3) special needs are—a good encyclopedia, bibliographical aids, such as the Zoölogical Record after 1893, the International Catalogue sections on botany, physiology, etc., from the beginning, The Royal Society Catalogue of Scientific Papers; and, finally, much more room is needed for the growth of the Library, and we are looking forward to this need being satisfied in the near future.

R. P. Bigelow,
Librarian.

VI. THE DIRECTOR'S REPORT.

To the Trustees of the Marine Biological Laboratory.

Gentlemen: I beg to present herewith a report of the thirty-fourth session of the Marine Biological Laboratory for the year 1921.

The session was in every sense a successful one; the attendance, which was far in excess of any previous year in the number of investigators, confirms again the status of the Laboratory as an effective organization for the promotion of biological research. The student attendance was the same as the preceding year, 120 in all courses, a number which has been fixed as a maximum with our present accommodations. The increase of the fee for courses from $50.00 to $75.00 first put into effect in 1921 thus did not operate to reduce attendance.

One hundred and seventy-two investigators, 36 more than in any preceding year, worked at the Laboratory. This was rendered possible by the completion of nine additional rooms in the brick building, with aid from the Nela Research Laboratory and Eli Lilly and Company acknowledged in the preceding report, by the use of five additional rooms in the newly completed laboratory of the Supply Department, and by assignments to the Laboratory of the Bureau of Fisheries at Wood's Hole generously placed at our disposal by the Commissioner, Dr. Hugh M. Smith. It would have been impossible to accommodate so many workers in the regular laboratories.

Fifty-two universities and colleges and research institutions subscribed for research rooms, or investigators' or students' tables (see appended list). This was the same number as in 1920, but the amount subscribed increased from $6,210.00 in 1920 to $8,800.00 in 1921. The total number of institutions represented by workers at the Laboratory was 95 scattered throughout practically all of the Eastern and Middle Western States. Workers from England, Holland, Russia, China, and Japan represented a growing international interest in the Laboratory.

Medical interests were represented by forty-one investigators and five students from twenty-seven medical institutions of this country, Canada, England, and China. When it is considered what a high percentage of other investigators on the side of animal biology are concerned in teaching and investigation of pre-medical subjects, it is fair to say that the relation of the Laboratory to medical interests is very close indeed. This is, of course, not a new situation in the Laboratory, but it is one that is developing strength year by year.

It is probably worth while at this time to give a summary comparison of the finances of the Laboratory for the last six years, because the financing of educational institutions has been notoriously difficult owing to rising costs, and most readers of the report will not take the time to compare the detailed reports of the treasurer for successive years. The following table gives a summary of the chief items of interest for the last six years:

	Expense.	Earned Income.	Donations for Current Expense.	Surplus without Depreciation.
1916......	$ 68,002.65	$52,776.87	$20,000.00	$ 4,774.22
1917......	69,753.70	52,504.85	20,000.00	2,751.15
1918......	69,687.14	54,282.05	20,000.00	4,594.91
1919......	88,452.64	80,307.57	20,260.00	12,114.93
1920......	103,097.64	89,943.04	20,060.00	6,905.40
1921......	109,970.40	98,292.38	23,148.00	11,469.28

	Assets.	Liabilities.	Reserve for Depreciation.	Trust Funds.
1916......	$329,845.93	$19,503.66	$ 6,942.12	$ 8,764.55
1917......	382,943.30	19,500.40	8,881.82	9,285.59
1918......	359,902.79[1]	17,019.79	21,140.24	9,954.03
1919......	376,307.31	20,429.21	28,543.46	10,583.41
1920......	411,123.63	27,263.50	34,799.48	10,409.66
1921......	430,251.28	31,051.72	42,070.17	11,004.44

It will be observed that, in spite of the great increases in cost of operation (expense) during this time, the earned income has maintained a very satisfactory ratio, so that the fairly constant donation account has left a "surplus" each year, if such it may be called; this has all been used, together with the special gifts, acknowledged in the reports, for the additions and improvements that have been found necessary, such as rebuilding the mess hall, erection of the

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[1] Decrease due to separate setting up of Gansett property account.

Supply Department Laboratory, completion of the brick building, increases of equipment, and in other minor ways, all of which are reflected in the increase of assets. The liabilities are made up for this table of the sum of accounts and notes payable, mortgage on the Gansett property and various lesser amounts, varying from year to year. The increase to be noted since 1918 is due mainly to loans amounting to $10,000 necessary to complete the heavy building and improvement operations of the last three years. The reserve for depreciation is merely a book charge diminishing the assets by the stated amount. The income of the small Trust Funds is allowed to accumulate, for the most part, and this accounts for their increase from year to year.

The treasurer's report for the current year (p. 279) reflects a satisfactory state of affairs so far as current expenses go. The increase of income over 1920, amounting to $8,349.34, is due to increase in receipts from instruction (from $5,960.00 in 1920 to $8,882.50 in 1921), from contributions for research rooms and tables ($4,450.00 and $6,325.00 in 1920 and 1921, respectively), from "Sundry Income," made up mostly of a charge of 6 per cent. on the investments in the mess, supply department, and dormitories ($7,030.75 and $9,243.76 in 1920 and 1921, respectively), and an increase of a little over $1,000.00 in the receipts from the mess. The receipts of the Supply Department, on the other hand, were nearly $1,000.00 less in 1921 than in 1920, which is, after all, a surprisingly small reduction in the face of the very adverse business conditions.

The new laboratory of the Supply Department and the additional rooms in the brick building were completed early in 1921 and were in use during the summer season.

Renewed and very careful consideration was given to the plans for the new buildings proposed in the reports for 1919 and 1920 and to means of securing funds for their construction and maintenance. A decision was soon reached to combine the three main needs, viz., a laboratory for biophysics and biochemistry, an auditorium, and library, in one building continuous with the present brick building, in order to use the land more economically, to simplify care of the buildings, and make the various departments more accessible to all workers; at the same time the plan provided a quiet corner for the auditorium in the angle between the two wings protecting it completely from the noise of the street. In their work on these plans the Executive Committee were able to rely on the advice and assistance of Mr. Charles A. Coolidge, at every stage, as in the plans studied in the summer of 1920. Drawings were made by Messrs. Coolidge and Shattuck of Boston and were repeatedly criticized and improved, detail by detail, by the workers at the Laboratory. The plans reached an advanced state of completion by the end of the summer, and the architects were then instructed to prepare final drawings and specifications and to secure contractors' estimates on the cost of construction.

During the year also efforts were continued with the aid of the National Research Council to secure the necessary funds for the building and its endowment, estimated at about one million dollars. The project was presented to the Rockefeller Foundation and the Carnegie Corporation by officers of the National Research Council early in the year, and opportunity was given during the session by both organizations for a full presentation of the needs and plans of the Laboratory by our Executive Committee. Both organizations had the matter under favorable consideration at the end of the year.

We are not asking for a large endowment in connection with the new building, but for only enough so that the operation of the new addition should not be unduly burdensome. Our aim should be to develop and extend the system of contributions from research sources of all kinds and to expand the business of the Supply Department and of all other sources of revenue. The Laboratory should depend largely upon coöperative support, to the least possible extent upon endowment. The morale and strength of the organization depend upon the necessity of its members working together for its support and its growth. The Laboratory will thus best maintain its ideals and become increasingly effective in the advancement of science.

The membership of our Corporation and Board of Trustees is composed almost entirely of scientific men; such a body is not usually considered well qualified to deal with matters of investment connected with relatively large endowments. On the other hand, to create another body, as an integral part of the Laboratory, that would have control of finances, would inevitably weaken the control of the scientific men over the policies of the Laboratory in general. Our Board of Trustees for these reasons adopted the policy that any endowment that should be created in connection with the present undertaking should be held in trust for the Laboratory by an outside body, which should pay in the income to the Laboratory for operating expenses.

If we realize these immediate aims within the next year or two, other practical problems will arise. The most serious of these probably is the housing problem which will be created by the increased attendance. This will be an important limiting factor in the growth of the Laboratory. There should be added a building capable of accommodating about 50 persons in separate rooms or small suites, situated as close to the Laboratory buildings and mess hall as possible. We should also erect about ten small bungalows, furnished and equipped for light housekeeping, on the Gansett tract, to accommodate some of our married members who can not now find reasonably priced and suitable accommodations for their families. About $75,000.00 is needed for these purposes. The rentals would furnish an addition to the income of the Laboratory, though it could not be expected to yield a high rate of return on the investment.

At the invitation of the Director, the Committee on Physiological Optics of the National Research Council held a session at the Laboratory from July 19 to July 22, inclusive. Advantage was taken of this occasion to hold several conferences on the general subject of biophysics between the members of the committee and investigators at the Laboratory. Demonstrations of biophysical investigations in progress were also held, and the sessions culminated in a symposium on "The Requirements of Biologists from Physical Science and on the Equipment of a Biophysical Laboratory." Numerous suggestions were received by the officers of the Laboratory for incorporation in the new plans. As one of the very few occasions in which the border-line problems between physics and biology have been discussed by specialists on both sides, the occasion was noteworthy. It should be regarded merely as a beginning of fruitful coöperation in biophysical research.

At the meeting of the Corporation August 9, 1921, four new members were elected to the Board of Trustees in the class of 1925: Professor I. F. Lewis, of the University of Virginia; Dr. R. S. Lillie, of the Nela Research Laboratories; Professor E. P. Lyon, of the University of Minnesota, and Professor D. H. Tennent, of Bryn Mawr College.

As part of their report to the Carnegie Corporation the Executive Committee drafted a statement concerning the Organization and Policies of the Marine Biological Laboratory. As this is the first attempt at such a formulation, it is herewith printed (p. 304) as part of the Director's report for the criticism of the members of the Laboratory. It is not intended to constitute a set of regulations. It is descriptive of the practices that have grown up in the past thirty-four years and of the traditions that have been established; thirty-four years hence such a statement may have considerable interest, after those who were concerned in the early history of the Laboratory shall have departed from this scene.

There are appended as parts of this report: (1) A list of the staff for 1921, (2) A list of investigators and students in attendance in 1921, (3) A tabular view of attendance for the last five years, (4) A list of the subscribing and coöperating institutions in 1921, (5) The Evening Lectures, (6) A statement concerning the Organization and Policies of the Marine Biological Laboratory, (7) A list of members of the Corporation.

I. THE STAFF.

1921.

Frank R. Lillie, *Director,* Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.
Gilman A. Drew, *Assistant Director,* Marine Biological Laboratory.

ZOÖLOGY.

I. INVESTIGATION.

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
E. G. Conklin, Professor of Zoölogy, Princeton University.
Gilman A. Drew, Assistant Director, Marine Biological Laboratory.
George Lefevre, Professor of Zoölogy, The University of Missouri.

Frank R. Lillie, Professor of Embryology, The University of Chicago.

C. E. McClung, Professor of Zoölogy, University of Pennsylvania.

T. H. Morgan, Professor of Experimental Zoölogy, Columbia University.

E. B. Wilson, Professor of Zoölogy, Columbia University.

II. INSTRUCTION.

W. C. Allee, Professor of Biology, Lake Forest College.

Edward J. Adolph, Instructor in General Physiology, University of Pittsburgh.

Robert Hall Bowen, Instructor in Zoölogy, Columbia University.

J. A. Dawson, Professor of Biology, Dalhousie University.

C. L. Parmenter, Instructor in Zoölogy, University of Pennsylvania.

Christianna Smith, Instructor in Zoölogy, Mount Holyoke College.

J. P. Visscher, Instructor in Zoölogy, Washington University.

Donnell B. Young, Assistant Professor of Zöology, Carlton College.

PROTOZOOLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

Gary N. Calkins, Professor of Protozoölogy, Columbia University.

Louise H. Gregory, Assistant Professor of Zoölogy, Columbia University.

EMBRYOLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

David H. Tennent, Professor of Biology, Bryn Mawr College.

Hubert B. Goodrich, Associate Professor of Zoölogy, Wesleyan University.

Benjamin H. Grave, Professor of Biology, Wabash College.

Charles G. Rogers, Professor of Comparative Physiology, Oberlin College.

Elizabeth A. Smith, Assistant Professor of Zoölogy, University of Wisconsin.

PHYSIOLOGY.

I. INVESTIGATION.

Albert P. Mathews, Professor of Biochemistry, The University of Cincinnati.

Harold C. Bradley, Assistant Professor of Physiological Chemistry, University of Wisconsin.

Walter E. Garrey, Professor of Physiology, Tulane University.

Ralph S. Lillie, Biologist, Nela Research Laboratory, Department of Pure Science, Nela Park, Cleveland, Ohio.

II. INSTRUCTION.

Ralph S. Lillie, Biologist, Nela Research Laboratory, Department of Pure Science, Nela Park, Cleveland, Ohio.

Merkel H. Jacobs, Assistant Professor of Zoölogy, University of Pennsylvania.

Frank P. Knowlton, Professor of Physiology, Syracuse University.

Reynold A. Spaeth, Associate in Physiology, School of Hygiene and Public Health, Johns Hopkins University.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES.

LECTURER.

Edward G. Spaulding, Professor of Philosophy, Princeton University.

BOTANY.

I. INVESTIGATION.

S. C. Brooks, Department of Public Health, Washington, D. C.

Edward M. East, Professor of Experimental Plant Morphology, Harvard University.

Robert A. Harper, Professor of Botany, Columbia University.

E. Newton Harvey, Assistant Professor of Physiology, Princeton University.

Winthrop J. V. Osterhout, Professor of Botany, Harvard University.

II. INSTRUCTION.

Ivey F. Lewis, Professor of Biology, University of Virginia.

Jacob R. Schramm, Professor of Botany, College of Agriculture, Cornell University.

William Randolph Taylor, Instructor in Botany, University of Pennsylvania.

LIBRARY.

Robert P. Bigelow, Librarian and Associate Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology. Librarian.

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Assistant Librarian.

CHEMICAL SUPPLIES.*

Oliver S. Strong, Associate Professor of Neurology, Columbia University, New York City, Chemist.

SUPPLY DEPARTMENT.

G. M. Gray, Curator.
John J. Veeder, Captain.
E. M. Lewis, Engineer.
A. W. Leathers, Head of Shipping Department.
A. M. Hilton, Collector.
J. McInnis, Collector.

F. M. MacNaught, Business Manager.

Herbert A. Hilton, Superintendent of Building and Grounds.

2. INVESTIGATORS AND STUDENTS—1921.

INDEPENDENT INVESTIGATORS—Zoölogy.

Addison, William H. F., Professor of Histology and Embryology, University of Pennsylvania.

Adolph, Edward F., University of Pittsburgh.

Allee, Warder C., Assistant Professor of Zoölogy, University of Chicago.

Allen, Ezra, Head of Biology Department, Ursinus College.

Baker, Horace B., Instructor, University of Pennsylvania.

Bigelow, Robert P., Associate Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology.

Boring, Alice M., Associate Professor of Zoölogy, Wellesley College.

Bowen, Robert H., Instructor in Zoölogy, Columbia University.

Calkins, Gary N., Professor of Protozoölogy, Columbia University.

Carothers, Eleanor E., University of Pennsylvania.

Carroll, Mitchell, Professor of Biology, Franklin and Marshall College.

* Compound microscopes, with two oculars and two objectives, rack and pinion and fine adjustment, may be rented for the season at $7.00 each, provided notice is received by the Business Manager not later than June 1. Microscopes are not otherwise supplied by the Laboratory.

Chambers, Robert, Assistant Professor of Anatomy, Cornell University Medical College.

Charlton, Harry H., Assistant Professor, University of Missouri.

Chidester, Floyd E., Associate Professor of Zoölogy, West Virginia University.

Clapp, Cornelia M., Professor Emeritus of Zoölogy, Mount Holyoke College.

Clark, Eliot R., Professor of Anatomy, University of Missouri.

Clark, Eleanor L., Columbia, Mo.

Copeland, Manton, Professor of Biology, Bowdoin College.

Cowdry, E. V., Associate Member, Rockefeller Institute.

Danchakoff, Vera, Assistant Professor of Anatomy, College of Physicians and Surgeons.

Danforth, Charles H., Associate Professor of Anatomy, Washington University School of Medicine.

Dart, Raymond A., Demonstrator of Anatomy, University of London.

Dawson, James A.. Professor of Biology, Dalhousie University.

Donaldson, Henry H., Professor of Neurology, Wistar Institute.

Drew, Gilman A., Assistant Director, Marine Biological Laboratory, Woods Hole, Mass.

Galtsoff, Paul S., 278 Hawthorne Ave., Yonkers, N. Y.

Glaser, Otto C., Professor of Biology, Amherst College.

Goodrich, H. B., Associate Professor of Zoölogy, Wesleyan University.

Grave, Caswell, Professor of Zoölogy, Washington University.

Grave, Benjamin H., Professor of Zoölogy, Wabash College.

Gregory, Louise H., Assistant Professor of Zoölogy, Barnard College.

Hausman, Leon A., Instructor in Biology and Protozoölogy, Cornell University.

Hemmeter, John C., Professor, University of Maryland.

Heilbrunn, Lewis V., Assistant Professor of Zoölogy, University of Michigan.

Henshaw, Henry W., Washington, D. C.

Hess, Walter N., Professor of Zoölogy, DePauw University.

Hogue, Mary J., Instructor, Johns Hopkins University Medical School.

Hoskins, Margaret M., Medical College, University of Virginia.

Howe, Harrison E., Chairman Division Research Extension, National Research Council, Washington, D. C.

Jacobs, Merkel H., Assistant Professor of Zoölogy, University of Pennsylvania.

Jennings, Herbert S., Professor of Zoölogy, Johns Hopkins University.

Just, Ernest E., Professor of Zoölogy, Howard University.

Kindred, James E., Assistant Professor of Biology, Western Reserve University.

Knower, Henry McE., Professor of Anatomy, University of Cincinnati.

Lefevre, George, Professor of Zoölogy, University of Missouri.

Lillie, Frank R., Chairman. Department of Zoölogy, University of Chicago.

Linton, Edwin, Honorary Fellow in Zoölogy, University of Missouri.

MacCallum, G. A., 925 St. Paul St., Baltimore, Md.

McClung, C. E., Director of Zoölogical Laboratory, University of Pennsylvania.

Martin, Bertha E., Associate Professor of Zoölogy, Mount Holyoke College.

Mast, Samuel O., Professor of Zoölogy, Johns Hopkins University.
Medes, Grace, Assistant Professor, Vassar College.
Miner, Roy W., American Museum of Natural History, New York City.
Morrill, Charles V., Assistant Professor of Anatomy, Cornell University Medical College.
Okkelberg, Peter, Assistant Professor of Zoölogy, University of Michigan.
Packard, Charles, Assistant Professor of Biology, Peking Union Medical College.
Parmenter, Charles L., Instructor, University of Pennsylvania.
Patten, William, Professor of Biology, Dartmouth College.
Peebles, Florence, Bryn Mawr, Pa.
Pinney, Mary E., Professor of Biology, Lake Erie College.
Plough, Harold H., Associate Professor of Biology, Amherst College.
Reese, Albert M., Professor of Zoölogy, West Virginia University.
Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.
Schrader, Franz, Bryn Mawr College.
Shellshear, Joseph L., Demonstrator of Anatomy, University of London.
Slosson, Edwin S., Editor of Science Service, Washington, D. C.
Smith, Elizabeth A., Assistant Professor of Zoölogy, University of Wisconsin.
Spaulding, Edward G., Princeton University.
Speidel, Carl C., Adjunct Professor of Anatomy, University of Virginia.
Stockard, Charles R., Professor of Anatomy, Cornell University Medical College.
Strong, Oliver S., Associate Professor of Neurology, Columbia University.
Swingle, Wilton W., Assistant Professor of Zoölogy, Yale University.
Tennent, David H., Professor of Biology, Bryn Mawr College.
Tracy, Henry C., Professor of Anatomy, University of Kansas.
Whedon, Arthur D., Instructor in Zoölogy, University of Pennsylvania.
White, Gertrude M., Instructor, Carnegie Institute of Technology.
Whiteside, Beatrice, 122 West 79th St., New York City, N. Y.
Whiting, Phineas W., Professor of Biology, St. Stephens College.
Wood, F. E., Professor of Biology, Illinois Wesleyan University.

BEGINNING INVESTIGATORS—Zoölogy.

Atterbury, Ruth R., Instructor, College of Physicians and Surgeons, New York City.
Bean, Raymond J., Instructor in Biology, Western Reserve University.
Chace, Eunice E., Instructor, Smith College.
Dellinger, Samuel C., Assistant Professor, University of Arkansas.
Geizer, Samuel W., Bruce Fellow, Johns Hopkins University.
Gilson, Arthur S., Jr., Harvard University.
Guthrie, Mary J., Bryn Mawr College.
Hayden, Margaret A., Instructor in Zoölogy, Wellesley College.
Hibbard, Hope, Assistant Professor of Biology, Elmira College.
Hinrichs, Marie A., Instructor, Vassar College.
Hoadley, Leigh, University of Chicago.
Johnson, George E., Professor of Biology, University of Porto Rico.

Lowther, Florence deL., Instructor, Barnard College.
McCosh, Gladys K., Assistant in Zoölogy, Washington University.
McQuinn, Lenore, Graduate Student, University of Michigan.
Matayoshi, Zenko, Assistant in Anatomy, Cornell University Medical College.
Ohshima, Hiroshi, Assistant Professor, Kyushu Imperial University.
Richter, Maurice N., 1019 Trinity Ave., New York City.
Romer, Alfred S., Instructor, Bellevue Medical College.
Sheppard, Alice E., 722 King St., Pottstown, Pa.
Sivickis, P., Fellow, Zoölogy Department, University of Chicago.
Smith, Christianna, Instructor, Mount Holyoke College.
Springer, Mary G., Assistant in Zoölogy, Oberlin College.
Swett, Francis H., Assistant in Zoölogy, Yale University.
Tharaldsen, Conrad E., Professor of Zoölogy, Northwestern University
Tyree, Dora, Assistant in Histology, University of Cincinnati.
Uhlemeyer, Bertha, Instructor in Zoölogy, Washington University.
Varian, Basil B., Columbia University.
Visscher, J. Paul, Instructor, Washington University.
Warren, Herbert S., Assistant in Zoölogy, Columbia University.
Whiting, Mrs. Phineas W., Annandale-on-Hudson, New York.
Woodbridge, Helen, Assistant in Zoölogy, Washington University.
Wyman, Leland C., Austin Teaching Fellow, Harvard University.
Young, Donnell B., Assistant Professor of Zoölogy, Carleton College.

INDEPENDENT INVESTIGATORS—Physiology.

Bradley, Harold C., Professor of Physiological Chemistry, University of Wisconsin.
Clowes, George H. A., Director of Research Division, Eli Lilly & Co., Indianapolis.
Collett, Mary E., Instructor in Physiology, University of Buffalo.
Collip, James B., University of Alberta, Alberta, Canada.
Davis, Alice R., 19 Ash St., Cambridge, Mass.
Edwards, Dayton J., Assistant Professor of Physiology, Cornell University Medical College.
Garrey, Walter E., Professor of Physiology, Tulane University.
Harvey, Mrs. E. N., Princeton, N. J.
Harvey, Dr. E. Newton, Professor of Physiology, Princeton University.
Hecht, Selig, Assistant Professor of Physiology, Creighton Medical College.
Knowlton, Frank P., Professor of Phsiology, Syracuse University.
Lillie, Ralph S., Biologist, Nela Research Laboratory, Cleveland, Ohio.
Loeb, Jacques, Head of Division of Experimental Biology, Rockefeller Institute for Medical Research.
Loeb, Leo, Professor of Comparative Pathology, Washington University.
Lyon, Elias P., Professor of Physiology, University of Minnesota.
Mathews, Albert P., Professor of Biochemistry, University of Cincinnati.
Moore, Arthur R., Professor of Physiology, Rutgers College.
Morgulis, Sergius, Professor of Physiological Chemistry, University of Nebraska, College of Medicine.
Morse, Withrow, Professor of Biochemistry, West Virginia University.

Pond, Samuel E., Physiologist, Nela Research Laboratory, Cleveland.

Sevringhaus, Elmer L., Instructor in Physiological Chemistry, University of Wisconsin.

Snyder, Charles D., Professor of Experimental Physiology, Johns Hopkins University.

Spaeth, Reynold A., Associate in Physiology, School of Public Health, Johns Hopkins University.

Van der Heyde, Henri C., Heemstede, Holland.

Warren, Howard C., Professor of Psychology, Princeton University.

Woodward, Alvalyn E., Demonstrator in Biology, Amherst College.

BEGINNING INVESTIGATORS—Physiology.

Bachman, Eda, Research Chemist, Eli Lilly & Co., Indianapolis.

Baskervill, Margaret L., Research Assistant, Nela Research Laboratory, Cleveland, Ohio.

Bills, Charles E., Graduate Student, Johns Hopkins University.

Blanchard, Kenneth C., Assistant in Biology, Clark University.

Cattell, Ware, Student, Cornell University.

Ni Tsang, G., University of Michigan.

Page, Irvine H., Chemist, Eli Lilly & Co., Indianapolis.

Sampson, Myra M., Assistant Professor of Zoölogy, Smith College.

Smith, Homer W., Eli Lilly & Co., Indianapolis.

Smith, Carlotta G., Eli Lilly & Co., Indianapolis.

Wollf, Nat S., Rochester, N. Y.

INDEPENDENT INVESTIGATORS—Botany.

Brooks, Sumner C., Hygienic Laboratory, United States Public Health Service.

Brooks, Mrs. S. C., Hygienic Laboratory, United States Public Health Service.

Gates, Reginald R., Professor of Botany, Kings College, University of London.

Harper, R. A., Professor of Botany, Columbia University.

Hazen, Tracy E., Assistant Professor of Botany, Columbia University.

Inman, O. L., Harvard University.

Jones, Lewis R., Professor of Plant Pathology, University of Wisconsin.

Lewis, Ivey F., Professor of Biology, University of Virginia.

Moore, George T., Director Missouri Botanical Garden, St. Louis, Mo.

Osterhout, Winthrop J. V., Professor of Botany, Harvard University.

Phillips, Everett F., Apiculturist, Department of Agriculture, Washington.

Schramm, J. R., Professor of Botany, Cornell University.

Taylor, William R., Instructor in Botany, University of Pennsylvania.

BEGINNING INVESTIGATORS—Botany.

DeBord, George G., Research Fellow in Hygiene, Harvard Medical School.

Gershay, Alexander, Assistant in Botany, Columbia University.

Hutchinson, Dorothy M., Auburn, Maine.

Kelley, Arthur P., Assistant in Botany, University of Pennsylvania.

MacInnes, Frances J., Massachusetts Institute of Technology.

Meneses, Laura, Radcliffe College.

Muenscher, W. C., Instructor, Cornell University.
Ray, George B., Teaching Fellow, Harvard University.
Smith, Edith P., Edinburgh, Scotland.

STUDENTS.

1921.

ZOÖLOGY.

Abbott, Elizabeth T., Vassar College.
Adcock, Florence G., Student, Knox College.
Allan, Isabelle, Hunter College.
Anastasia, Carmela, Student, Connecticut College.
Atlee, John L., Jr., Student, Franklin and Marshall College.
Benedix, Agnes W., Teacher, Hunter College High School.
Bennet, Agnes H., Student, Barnard College.
Bollman, Loretta M., Instructor, Notre Dame College.
Brown, Frances T., Oberlin College.
Brown, Roland W., Instructor, Pennsylvania Forestry School.
Bullock, Emily W., Laboratory Assistant, Mount Holyoke College.
Calef, Dorothy A., University of Chicago.
Chase, Ruth W., Assistant in Zoölogy, University of Wisconsin.
Cornell, Mildred, Student, Knox College.
Council, Frank E., Instructor, Texas Christian University.
Davidson, John G., Principal, Delmont High School.
Emmart, Emily W., Student, Goucher College.
Everett, M. Gertrude, Student, Knox College.
Fisher, Mary J., Instructor in Zoölogy, Cornell University.
Flexner, Louis B., Student, University of Chicago.
Geer, Gertrude H., Student, Sweet Briar College.
Going, Ellen M., 130 McTavish St., Montreal, Canada.
Gregg, Mabel J., Assistant in Zoölogy, University of Wisconsin.
Gregory, Allene, Washington, D. C.
Huber, Charles B., Student, Franklin and Marshall College.
Husted, Glenn, Student, Illinois Wesleyan University.
Ives, Florence B., Sweet Briar College.
Johnson, Alfred M., Student, Wabash College.
Jordan, Edwin P., Student, University of Chicago.
Joy, Edward A., Instructor in Biology, Tufts College.
Lincoln, N. Stanley, Student, Wesleyan University.
McCaa, Fanny, Assistant Instructor, Agnes Scott College.
McCarty, Sarah H., Student, Barnard College.
McGavran, Edward G., Student, Butler College.
Markee. Lois E., Student, Knox College.
Masbach, Henrietta, Student, Goucher College.
Medwedeff, Minnie V., Goucher College.

Murray, Margaret R., Student, Goucher College.
Myers, Everett C., Student, Adelbert College, Western Reserve University.
Nichols, Elizabeth, Student, Agnes Scott College.
Nichols, Rhoda, Student, Wellesley College.
Pell, Orlie A. H., 112 East 74th St., New York City.
Scott, Helen M., Instructor, Illinois Wesleyan University.
Smith, Abigail E., Student, Wellesley College.
Stewart, Dorothy R., Mount Holyoke College.
Stoll, Ione, Student, Hunter College.
Unger, W. Byers, Instructor in Biology, Lafayette College.
Vreeland, Maebelle, Student, Western State Normal School.
Wicks, Nina A., Student, Knox College.
Wilson, Constance C., Student, Wellesley College.
Woodhead, Arthur E., Professor of Chemistry and Biology, Western Maryland College.
Woodward, Mildred, Student, Smith College.
Young, Benjamin P., Assistant Professor of Zoölogy, Cornell University.

PROTOZOÖLOGY.

Austin, Mary L., Laboratory Assistant in Zoölogy, Wellesley College.
Buck, Ruth, University of Missouri.
Burns, Robert K., Assistant in Zoölogy, Yale University.
Fry, Henry J., Assistant in Zoölogy Department, Columbia University.
Green, Wilmoth, Assistant in Zoölogy Laboratory, Washington University.
Leland, Stanley J., DePauw University.
MacDougall, Mary S., Head of Biology Department, Agnes Scott College.
Martinaglia, Giovanni, Rockefeller Institute, Princeton.
Meleney, Grace C., Columbia University.
Menninger William C., Cornell University Medical College.
Metcalf, Rachel V., Instructor, Mount Holyoke College.

EMBRYOLOGY.

Bishop, Mabel, Head of Department of Biology, Hood College.
Botsford, E. Frances, Instructor in Physiology, Mount Holyoke College.
Brailey, Miriam E., Student, Mount Holyoke College.
Bumpus, Laurin D., Student, Brown University.
Casey, Gertrude U., Student, Simmons College.
Dimick, Gertrude P., Student, Smith College.
Edington, Robert P., Student, Wabash College.
Gould, Alice, Technician in Public Health, Yale University.
Grant, Madeline P., Graduate Student, Radcliffe College.
Gray, Julius C., Williams College.
Hartwell, Rhoda A., Student, Mount Holyoke College.
Heaton, Louise M., Student, Mount Holyoke College.
Hill, Constance A., Student, Connecticut College.
Howard, Nelson J., Johns Hopkins University.
Klemm, Ralph A., Student, Haverford College.

Mall, Mary L., Johns Hopkins Medical School.
Martin, Earl A., Student, College of the City of New York.
McClay, Edith L., Cornell University Medical College.
Myer, George A., Student, Johns Hopkins University.
Nute, Bertha E., 53 Hotchkiss Place, Torrington, Conn.
O'Toole, George B., Professor of Philosophy, St. Vincent's Archabbey.
Stilwell, Frances, Student, Smith College.
Titus, Jessie, Assistant, Long Island College Hospital.
Uichanco, Leopoldo B., Fellow in Entomology, University of the Philippines.
Warren, William A., Student, Dartmouth College.
Waterhouse, Isabel R., Student, Mount Holyoke College.
Williams, S. Culver, Wesleyan University.
Yolton, Leroy W., Illinois Wesleyan University.

PHYSIOLOGY.

Barnes, Hilda, Student, Smith College.
Brown, Alice L., Instructor in Zoölogy, Kansas State Agricultural College.
Chadbourne, Emily R., Bryn Mawr College.
Hausmann, Theo. W., Instructor in Science, Concordia Collegiate Institute.
Hoffman, Hannah G., Student, Barnard College.
Hultman, Jennie E., Teacher of Biology, Omaha.
Lowman, Edith, Student, Vassar College.
Millikin, Neil, Student, Johns Hopkins University.
Oslund, Robert, University of Chicago.
Pearse, Herman E., Jr., Student, University of Missouri.
Pinkerton, Florence A., Student, Simmons College.
Rathbone, Josephine L., Wellesley College.
Tilden, Evelyn B., Rockefeller Institute for Medical Research.
Walters, Mary J., Goucher College.
Webster, Eleanor F., Student, University of Pennsylvania.
Wong, A. D., Oberlin College.

Attending Morning Lectures only.

Harper, Francis D., 5728 Woodlawn Ave., Chicago, Ill.
Van Duyne, S. Elizabeth, Assistant Professor of Physiology and Hygiene, Goucher College.

BOTANY.

Adolph, Mary B., 3408 Wallace St., Philadelphia.
Chadbourne, Elisabeth, Student, Smith College.
Ferguson, George W., Student, Dartmouth College.
Hunt, Catherine T., Teacher, West Roxbury, Mass.
Hylander, Clarence J., Student, Yale University.
Keefe, Anselm, St. Norbert's College.
Kemp, Margaret, Student, Smith College.
Lackey, James B., Jackson, Miss.
MacCreight, Jean, 1358 Marlborough St., Philadelphia.
Mitchell, Esther, Student, Vassar College.

3. TABULAR VIEW OF ATTENDANCE.

	1917	1918	1919	1920	1921
INVESTIGATORS—Total	129	93	134	136	172
Independent:					
Zoölogy	63	51	68	69	79
Physiology	23	16	24	22	26
Botany	8	5	7	7	13
Under Instruction:					
Zoölogy	24	16	21	29	34
Physiology	6	3	10	7	11
Botany	5	2	4	2	9
STUDENTS—Total	83	69	128	120	120
Zoölogy	46	41	55	56	53
Protozoölogy	—	—	15	15	11
Embryology	16	12	33	26	28
Physiology	13	10	17	15	18
Botany	8	6	8	8	10
TOTAL ATTENDANCE	212	162	262	256	292
INSTITUTIONS REPRESENTED—Total	77	72	88	86	95
By investigators	60	49	61	55	67
By students	36	38	62	57	53
SCHOOLS AND ACADEMIES REPRESENTED.					
By investigators	2	—	—	1	—
By students	5	—	4	7	—

4. SUBSCRIBING AND COOPERATING INSTITUTIONS IN 1921.

Barnard College
Bowdoin College
Bryn Mawr College
Butler College
College of Physicians and Surgeons
Columbia University
Connecticut College
Cornell University
Cornell University Medical College
Creighton University
Dartmouth College
Eli Lilly & Co.
Federal Board of Vocational Education
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Illinois Wesleyan University
Johns Hopkins University
Knox College
Massachusetts Institute of Technology
Mount Holyoke College
Nela Research Laboratory
Oberlin College
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Simmons College
Smith College
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Kansas
University of Michigan
University of Missouri
University of Nebraska
University of Pennsylvania
University of Philippines
University of Virginia
University of Wisconsin
Vassar College
Wabash College
Washington University
Wesleyan University
Wellesley College
Western Reserve University
Western State Normal School
Williams College
Wistar Institute of Anatomy and Biology
Yale University

Scholarship Tables.

The Lucretia Crocker Scholarships for Teachers in Boston, Since 1888.

Scholarship of $100.00, Supported by a Friend of the Laboratory, Since 1898.

The New London Branch of the American Association of University Women, Since 1920.

5. EVENING LECTURES, 1921.

Friday, July 1,
Dr. W. J. V. Osterhout...... 'Injury and Recovery."
Tuesday, July 5,
Dr. E. R. Clark..............“The Reaction of Living Cells in the Tail of the Frog Larvæ to Foreign Substances."
Friday, July 8,
Dr. Gary N. Calkins......... “Differentiation and Vitality in Protozoa."
Tuesday, July 12,
Dr. R. S. Lillie.............. “The Conditions of Growth in Living and in Non-living Systems."
Friday, July 15,
Dr. Wallace O. Fenn......... “Phagocytosis."
Tuesday, July 19,
Dr. E. V. Cowdry............ “The Conflict between Old and New Medicine in China."
Friday, July 22,
Dr. G. H. A. Clowes......... “Some Physical Chemical Concepts of Protoplasmic Structure."
Tuesday, July 26,
Dr. H. H. Donaldson...........“Rat Studies."
Friday, July 29,
Dr. I. W. Bailey.............“The Significance of the Cambium in the Investigation of Certain Cytological and Physiological Problems."
Tuesday, August 2,
Mr. Wm. L. Underwood....... 'Adventures of a Sage-brush Tourist in Wyoming."
Friday, August 5,
Dr. E. E. Slosson............“History from the Standpoint of the Second Law of Thermo-dynamics."
Monday, August 8,
Dr. S. C. Brooks..............“Immunity and Ultra-violet Light."

6. THE ORGANIZATION AND POLICIES OF THE MARINE BIOLOGICAL LABORATORY.

The Marine Biological Laboratory is an outpost of the higher educational and research institutions of the country. Representatives of such institutions are eligible to membership in the controlling body, the Corporation of the Marine Biological Laboratory, composed of over 400 persons, mostly biologists. Institutions also have further control and interest through their ability for a moderate sum to use assigned quarters for members of their staff or student body. They may thus from year to year continue to occupy the same quarters and possess veritable extra-territorial control. The Laboratory actually belongs to the biologists and through them to their institutions. The function of the administration of the Laboratory is the execution of a trust held for common use.

The organization thus provides for a nominal sum facilities that would cost each institution many times the amount to provide independently. This is important even for the largest institutions, and indispensable for all the smaller institutions that aim to maintain the research spirit of members of their staffs.

1. *Organization.*—The Marine Biological Laboratory is an independent Corporation operating under its own charter. The act of incorporation of the Marine Biological Laboratory and the By-laws of the Corporation are printed in each of the Annual Reports. The membership of the Corporation and of the Board of Trustees is also printed in the same places. The Corporation meets on the second Tuesday in August each year at the Laboratory. At each annual meeting it elects eight trustees to serve four years, and a Treasurer and Clerk of the Corporation who are *ex officio* members of the Board of Trustees; the Director and Assistant Director, who are appointed by the Trustees, are also *ex officio* members of the Board.

The Trustees, who are mostly biologists selected for their scientific attainments, have the control and management of the affairs of the Corporation; they, on their part, delegate most of their functions to an executive committee of five members, including the Director and Assistant Director *ex officio.*

The membership of the Corporation represents all important phases of biological research and most American institutions in which such work is done. Any competent investigator qualified for admission to the principal national research societies in Biology is eligible for membership, which is also open to other persons willing and qualified to render useful service to the Corporation. (See rules of eligibility in the records of the Clerk of the Corporation.) The ultimate control and ownership of the Laboratory is vested in the Corporation.

2. *Purpose.*—The primary purpose of the Marine Biological Laboratory is the advancement of Biological Science. This is the first consideration in every matter of policy. The *scope* is conceived as broad as the field of biology, but the *aim* is to promote the fundamental and more general rather than the systematic and more special problems. The fundamental problems trace back to the cell, and are based ultimately on physics and chemistry; they thus require some of the special equipment of both of these fields. Considerations of pure science are primary and those of utility secondary, though recognized as important. Thus the work of the Laboratory bears important relations to medicine, agriculture, fisheries, and economic biology.

3. *Research.*—Individual research is the foundation of all advance in science. Hence all policies of the Laboratory are directed toward the investigator with the object of furnishing him opportunities, facilities, and freedom for research.

The Laboratory has no set program of research as an institution, but aims to be hospitable to all branches of biological science; it should thus reflect and stimulate progress in all directions, if not simultaneously, at least in the course of time.

It is the policy of the Laboratory to favor such forms of coöperation in research as develop naturally. The mere association of so many investigators as meet annually at the Laboratory inevitably develops coöperative relations of various kinds, and this is regarded as one of the most valuable results of the organization.

Investigators are admitted to the Marine Biological Laboratory on the judgment of the Director as to their fitness to make good use of the facilities offered.

Two classes of investigators are recognized: those who work independently, and those receiving instruction in investigation.

Investigators' fees are generally paid by the institution to which the investigator belongs, but it is at the discretion of the Director to admit any investigator without charge, and it has never been the policy to refuse admission to any independent investigator solely on the issue of a fee.

The more experienced investigators, or a selected group thereof, guide the research of beginning investigators. No group in the organization will be of more significance for the future of the Laboratory than beginners in research.

Admission to the Laboratory is not limited by considerations of nationality. It has become desirable to pursue a positive instead of a merely passive or negative attitude in the matter of international representation.

The Laboratory is open to investigators throughout the year.

The Laboratory is open on the same terms to both sexes.

4. *Instruction.*—In pursuit of the primary purpose of the Laboratory it is essential that provision should be made for the production and training of investigators. It is important that a certain part of such provision should be in the Laboratory itself. Hence instruction of a strictly professional character is also a settled policy of the Laboratory. Experience has shown that instruction may be overemphasized to the detriment of research; hence the policy of the Laboratory is to limit the courses of instruction offered (only five for many years), to require fees adequate to pay all the expenses of instruction, to limit the numbers registered in each course, and to admit no one without satisfactory recommendations.

The intellectual atmosphere of the Laboratory is favorable in the highest possible degree to the development of the research spirit in young students. For their own sake and for the sake of the future development of biology they should not be excluded from the Laboratory.

In the case of all courses offered, the facilities of the Laboratory, whether of personnel or material, are such as to guarantee that they may be offered to greater advantage here than elsewhere. There is no attempt to duplicate university or college courses.

The Laboratory assigns no grades in courses and sets no examinations. The Laboratory will therefore not certify to attainments in any course, but individual instructors are at liberty to do so on their own responsibility.

Instructors in courses are selected primarily for their interest in and qualifications for research.

Salaries of instructors are calculated to pay expenses of travel and living only for the duration of the course.

The number of instructors in courses is sufficient to leave each one adequate time for his own research.

Courses of instruction are given only during six weeks in the summer.

5. *Coöperating Institutions.*—The organization of the Laboratory, being primarily a coöperative society of biological investigators and of the institutions that they represent, is such that a system of subscribing and coöperating institutions has been gradually developed (see p. 302); these relationships should be further extended and strengthened.

As far as possible the same space is reserved in successive years for the coöperating institutions. But, except where definite contracts exist, the assignment of space is at the discretion of the Director.

The Laboratory aims to be an outpost of the biological departments of universities, colleges, and research laboratories; each has its part in the Laboratory according to its needs and acquired rights.

6. *Financial.*—In general, it is the aim of the Laboratory to make all departments except research self-supporting. The Laboratory should depend as little as possible upon actual endowment and as much as possible upon earnings and good will. It is the policy of the Laboratory to have endowment funds held in trust.

The financial administration should be such as to secure confidence; to this end there is a complete accounting system for all income and expenditures and an annual audit by a firm of auditors, which is published each year.

A budget estimate is prepared in December of each year for the year following and submitted to the Board of Trustees, or Executive Committee, for consideration.

7. *Staff.*—The Assistant Director and operating staff on annual appointments receive their livelihood from the Laboratory; the salaries should be ample in order to secure the best men.

The scientific staff on the research side serves without salary; salaries of instructors are calculated to pay expenses of travel and living for the duration of the course.

There is no permanent scientific staff. The establishment of such a staff would tend to endanger the principle of freedom of development for all branches of biology by favoring a few. It would also set the Laboratory in competition with universities and research institutions for men and weaken the spirit of coöperation of such institutions. It would favor a few individuals at the expense of the many.

8. *The Supply Department* serves primarily to obtain and distribute living materials for research within the institution; class supplies are also furnished. This service is furnished without additional fee to investigators and students. This is an essential part of the organization. It saves the time of the investigators and through intelligent collecting depletion of the grounds is safeguarded.

The major part of the work of the Supply Department is, however, to furnish biological supplies to institutions. It is the policy to develop this business as far as possible. The main advantages are: (1) The profits from sales are an important item of income. (2) The business is continuous throughout the year, and the Laboratory is thus enabled to keep together a permanent skilled staff of collectors. (3) A real service is rendered to biological education and research by providing a source of standardized and reliable material of many kinds. (4) This service constitutes an important item of good-will.

9. *The Department of Chemical Supplies.*—In general, it is the function of this department to furnish all standard apparatus and supplies ordinarily used in the work of investigation at the Laboratory with no additional charge. Exceptions are noted in the printed rules of the department. Microscopes, microtomes, and

"special" apparatus and reagents are not furnished. The policy is summed up in "service to the investigator."

10. *The Library.*—It is the policy of the Laboratory to develop the best possible library for current biological research. Books of historical interest only are regarded as secondary in importance. The development of this policy will involve the establishment of a very large library in the course of time, and this should be pursued as rapidly as possible.

11. *The Mess* is conducted for the benefit of investigators and students. Rates are calculated to pay the cost of operation, equipment, rent, and upkeep in general, and not for profit.

Rules and regulations have been established governing its use. These are subject to revision by the Executive Committee of the Laboratory at any time.

12. *Housing Facilities.*—The housing facilities furnished by the Laboratory are calculated to meet the deficit in the local supply and to keep local prices at a reasonable rate. As the local provision has become progressively less adequate, it has been necessary to make increasing provision for workers. The purchase of the Gansett property, twenty acres within a mile of the Laboratory, was determined by this consideration. The development of the Laboratory obviously depends upon adequate housing facilities at reasonable rates.

13. *Biological Bulletin.*—One of the policies of the Laboratory should be to aid in the provision of adequate means for publication of biological research in America. The establishment of the BIOLOGICAL BULLETIN in 1899 was in pursuit of this policy; it is now in its forty-first volume. A larger responsibility should be undertaken as soon as means permit.

14. *Future Development.*—The policy of attempting adequately to represent biological research in America by free association of, and government by, the investigators themselves determines all questions of future development. The next step will generally be apparent, as, for instance, the present need of a biophysical and biochemical laboratory, and of a building for library, administration, and auditorium. It is the policy of the Laboratory to be ready for advance by the nature and morale of its organization and by control of sufficient land.

7. MEMBERS OF THE CORPORATION.

1. Life Members.

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Carey, Mr. Arthur Astor, Fayerweather St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Farlow, Prof. W. G., Harvard University, Cambridge, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, 955 Park Ave., New York City, N. Y.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
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Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
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Mason, Miss Ida M., 1 Walnut St., Boston, Mass.
Means, Mr. James Howard, 196 Beacon St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.

Morgan, Prof. T. H., Columbia University, New York City, N. Y.

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Phillips, Dr. John C., Windy Knob, Wenham, Mass.

Phillips, Mrs. John C., Windy Knob, Wenham, Mass.

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Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.

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Harvey, Mrs. E. N., Princeton, N. J.

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Lillie, Prof. R. S., Nela Research Laboratory, Nela Park, Cleveland, Ohio.

Linton, Prof. Edwin, 1408 Rosemary Lane, Columbia, Mo.

Loeb, Prof. Jacques, Rockefeller Institute for Medical Research, New York City.

Loeb, Prof. Leo, Washington University Medical School, St. Louis, Mo.

Lowther, Mrs. Florence DeL., Barnard College, Columbia University, New York City.

Lund, Dr. E. J., University of Minnesota, Minneapolis, Minn.

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Lyman, Prof. George R., Plant Disease Survey, Department of Agriculture, Washington, D. C.

Lynch, Miss Clara J., 434 West 120th St., New York City.

Lyon, Prof. E. P., University of Minnesota, Minneapolis, Minn.

MacCallum, Dr. G. A., 925 St. Paul St., Baltimore, Md.

McClung, Prof. C. E., University of Pennsylvania, Philadelphia, Pa.

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McGill, Dr. Caroline, Murray Hospital, Butte, Montana.

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Martin, Miss Bertha E., Mount Holyoke College, South Hadley, Mass.

Mast, Prof. S. O., Johns Hopkins University, Baltimore, Md.

Mathews, Prof. A. P., University of Cincinnati, Cincinnati, Ohio.

Matsui, Prof. K., Imperial College of Agriculture and Dendrology, Morioka, Japan.

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Meigs, Dr. E. B., Dairy Division Experiment Station, Beltsville, Md.

Meigs, Mrs. E. B., 1445 Rhode Island Ave., Washington, D. C.

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Metz, Prof. Charles W., Carnegie Institution of Washington, Cold Spring Harbor, Long Island.

Miner, Dr. Roy W., American Museum of Natural History, New York City.

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Moore, Dr. A. R., Rutgers College, New Brunswick, N. J.

Moore, Miss Dorothea M., 21 Kirkland St., Cambridge, Mass.

Morgan, Prof. H. A., Agricultural Experiment Station, Knoxville, Tenn.

Morgan, Dr. Anna H., Mount Holyoke College, South Hadley, Mass.

Morrill, Prof. A. D., Hamilton College, Clinton, N. Y.

Morrill, Prof. C. V., Cornell University Medical College, New York City.

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Nabours, Dr. R. K., Kansas State Agricultural College, Manhattan, Kans.

Nachtrieb, Prof. Henry F., University of Minnesota, Minneapolis, Minn.

Neal, Prof. H. V., Tufts College, Tufts College, Mass.

Newman, Prof. H. H., University of Chicago, Chicago, Ill.

Nichols, Dr. M. Louise, 3221 Race St., Philadelphia, Pa.

Nonidez, Dr. Jose F., Cornell University Medical College, New York City.

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Pearse, Prof. A. S., University of Wisconsin, Madison, Wis.
Peebles, Prof. Florence, Bryn Mawr College, Bryn Mawr, Pa.
Phillips, Miss Ruth L., Western College, Oxford, Ohio.
Phillips, Dr. E. F., Department of Agriculture, Washington, D. C.
Pike, Prof. Frank H., 437 West 59th St., New York City, N. Y.
Pinney, Miss Mary E., Lake Erie College, Painesville, Ohio.
Plough, Prof. Harold H., Amherst College, Amherst, Mass.
Pond, Dr. Samuel E., Nela Research Laboratory, Nela Park, Cleveland, Ohio.
Porter, Mrs. M. B., 310 East 21st St., Austin, Texas.
Pratt, Dr. Frederick H., Boston University School of Medicine, Boston, Mass.
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Rankin, Prof. W. M., Princeton University, Princeton, N. J.
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Rogers, Prof. Charles G., Oberlin College, Oberlin, Ohio.
Romer, Dr. Alfred S., Columbia University, New York City.
Rudisch, Dr. J., Mount Sinai Hospital, New York City, N. Y.
Sampson, Miss Myra M., Smith College, Northampton, Mass.
Sands, Miss Adelaide G., 348 N. Main St., Port Chester, N. Y.
Sands, Dr. Georgiana, 348 N. Main St., Port Chester, N. Y.

Scott, Dr. Ernest L., Columbia University, New York City, N.Y.

Scott, Prof. G. G., College of the City of New York, New York City.

Scott, Prof. John W., University of Wyoming, Laramie, Wyo.

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Shumway, Dr. Waldo, Dartmouth College, Hanover, N. H.

Sivickis, Mr. P. B., University of Chicago, Chicago, Ill.

Smith, Dr. Bertram G., 416 West 118th St., New York City.

Snow, Miss Laetitia M., Wellesley College, Wellesley, Mass.

Snyder, Prof. Charles D., Johns Hopkins University, Baltimore, Md.

Sollmann, Dr. Torald, Western Reserve University, Cleveland, Ohio.

Spaeth, Dr. Reynold A., School of Public Health, Johns Hopkins University, Baltimore, Md.

Speidel, Dr. Carl C., University of Virginia, Charlottesville, Va.

Spencer, Prof. H. J., 123 Waverley Place, New York City.

Stockard, Prof. C. R., Cornell University Medical College, New York City.

Stokey, Dr. Alma G., Mount Holyoke College, South Hadley, Mass.

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WHITE, DR. E. GRACE, Shorter College, Rome, Ga.

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Williams, Dr. Anna W., 549 Riverside Drive, New York City, N. Y.
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STUDIES ON THE NECESSITY OF THE BRAIN FOR THE INCEPTION OF INSECT METAMORPHOSIS.

STEFAN KOPEĆ,

Government Institute for Agricultural Research, Pulawy, Poland.[1]

With 2 Tables and 4 Figures in Text.

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Gudernatsch ('12, '14) was the first to describe the acceleration of metamorphosis in tadpoles fed on thyroid. These results were confirmed for different amphibians by Laufberger ('13), Cotronei ('14), Brendgen ('14), Abderhalden ('15, '19), Kaufman ('17, '18), Kahn ('16), Hirschler ('18–'19), etc., and first of all by Romeis ('14–'19). Adler ('16) ascertained that changes of temperature of the environment cause changes of celerity of metamorphosis in tadpoles, together with simultaneous differences in the structure of the thyroid.

The results of the investigations of Loeb ('96) and Wintrebert ('05–'11), who cut the spinal cord or the nerves of larvæ, contradict the influence of the brain on metamorphosis of amphibians, or at least the influence by means of nerves. On the other hand, Babák ('05) examined the brain of the toad and came to the conclusion that the hind part of the brain affects metamorphosis, since, when it is removed, the processes are arrested. He lays stress on the fact that the removal of the brain effects the changes described only in case the animal is operated upon before the first pair of limbs have grown. Babák ('13) believes that here we have the influence of hypophysis by means of chemical stimuli. Gudernatsch ('12, '14) by feeding tadpoles on hypophysis did not bring about—it is true—any changes in the rate of development, but there is no doubt that feeding experiments with a certain organ may be considered as decisive only if their results are positive, a fact which is not well understood by certain investigators. In fact, Adler ('14), Smith ('16), and Allen ('16, '17) confirm that the processes of metamorphosis in tadpoles are arrested by removal of the hypophysis, which, however, is accompanied by changes in the thyroid. On the other hand, Rogers ('18) and Larson ('19) described changes of the hypophysis brought about by removal of the thyroid in tadpoles, and Allen ('18) confirmed the fact that tadpoles deprived of their thyroid do not undergo metamorphosis. The question arises, therefore, whether the essential and decisive factor which regulates the transformation in those animals is the function of the hypophysis or of the thyroid. Hirschler ('18–'19) ascertained that metamorphosis is accelerated by injecting axolotls with iodoform or iodin. From the recent researches made by Swingle ('19*a* and *b*), who succeeded in transforming tadpoles deprived of their thyroid by feeding them on iodin with flour, as well as from his own analogous experiments, Allen ('19) draws the general conclusion that the metamorphosis of amphibians depends on iodin stored by the thyroid, and that its secretory function is regulated by the hypophysis.

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[1] Paper from the Embryologico-Biological Laboratory, Jagellonian University, Cracow, Poland, presented in the Acad. of Sc., Cracow. *Cf.* Bull. Acad. d. Sc. Cracovie, 1917.

The relationship of the ganglia of the head to metamorphosis in insects has been studied by Conte and Vaney ('11). These authors made ligatures between the head and the body of caterpillars of *Lymantria dispar* L. and obtained adult moths. They infer from their experiments that the ganglia of the head are of no importance for these processes. The following pages show, however, that their conclusion is false. We shall see that the brain (ganglion supra-œsophageale) exerts a great influence on metamorphosis of insects, and the cause of the erroneous conclusion will be clearly explained.

If we wish to investigate the relation which probably exists between the nervous system and the processes of metamorphosis of insects, we must first of all decide whether, after the removal of single parts of the nervous system, the rate of metamorphosis is in general the same as in normal animals, whether it undergoes acceleration or retardation, or whether it is completely arrested. The problem was to find out a possibly well-defined center which would have a more or less decisive influence on the time of inception or on the rate of metamorphosis. Further, we must consider the means by which this hypothetical influence could act on the processes of metamorphosis. The mechanism of this influence ought to be studied by means of special experiments, and the time at which the influence begins to act ought to be found out. In order to answer this question, I operated on female caterpillars of *Lymantria dispar* L. after their last moult. Thus I was able to consider only the processes of the pupation of caterpillars and the emergence of the adult moths, without taking into consideration the processes of the larval moult.

It must be distinctly remarked that the whole material, experimental as well as that used as a control, came from eggs of one female only. All the caterpillars were reared in precisely identical conditions. Moulting, pupation, and emergence of moths were controlled every 12 hours; thus the error in determining the length of life of caterpillars and chrysalids (see Tables I. and II.) could not exceed 24 hours.

As to the methods of removal of single ganglia, cf. operations in my former paper (Kopeč, '18).

A. The Influence of the Brain on the Inception of the Processes of Metamorphosis.

The behavior of caterpillars of *Lymantria dispar* L. deprived of their brain (ganglion supra-œsophageale) allows us to draw the conclusion that this ganglion has a quite specific quality, very important to the whole organism, and characterizing the brain as an organ which excites histolytical processes in the caterpillar and regulates the time of inception of the general processes of pupation. Let us consider the data given in Table I., which refer to the pupation or to the death of caterpillars, whether normal, brainless, or those used as a control, viz., specimens which have been injured in the same manner as the caterpillars deprived of their brain, and which, in spite of the presence of the brain, refused food as well as the brainless specimens. All these specimens (exclusively females) were operated upon on the seventh day after their last moult and lived under the same conditions of heat, light, and humidity. We see that out of 25 brainless caterpillars only 5 underwent pupation, and in these the instant of beginning pupation exhibits a certain retardation in comparison with the behavior of the specimens used as a control. It is evident that the majority of the brainless caterpillars did not undergo metamorphosis, remained alive for weeks, and did not die until the whole store of fat in their body had been completely exhausted. Large full-blooded and fat caterpillars of 4–5 cm. in length become small during this period, and were often scarcely 1 cm. long in their final state. At the same time their bodies shrank and almost completely dried up, their movements became fainter and fainter, until at last they died. Caterpillars deprived of their brain do not, as a rule, die after the operation as a result of any disease induced by the removal of the brain, but, on the contrary, they live relatively very long. Only a few specimens die soon after operation as a consequence of mechanical injuries taking place during the removal of the brain. The essential difference between these insects and those used as a control lies only in the fact that histolytical processes subsequently followed by metamorphosis can begin only in animals the brain of which has exerted a proper stimulus at the proper time. It is certain that the few brainless caterpillars which succeeded in becoming pupæ attained the stagé only because the brain had already influenced the tissues before its removal. In this function, too, the animals exhibit individual fluctuations; here we evidently see specimens in which the brain had begun to perform its stimulative part very early, or had exerted it very quickly. It ought to be admitted that, if we could succeed in feeding brainless specimens, their life could be still longer. These caterpillars, however, would probably not undergo metamorphosis.

The opinion that the brain is of importance for metamorphosis is supported by the following observations, which prove also that the influence discussed sets in at a somewhat definite period, viz., in females of *Lymantria dispar* L. in my breed between the seventh and tenth day after their last moult. Let us observe the behavior of the caterpillars which were deprived of their brain not on the seventh day after the last moult as in investigations hitherto con-

TABLE I. BEHAVIOR OF THE FEMALE CATERPILLARS OPERATED UPON ON THE SEVENTH DAY AFTER THEIR LAST MOULT.
p. = pupation; d. = death of caterpillars.

Caterpillars Deprived of the Brain.			Caterpillars Deprived of the Subœsophageal Ganglion (Brain Intact).			Caterpillars Used as a Control; Injuries of the Head as in Caterpillars Deprived of Brain (Brain Intact).			Normal Caterpillars.		
Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.
1	After 12½ days p.	—	1	After 7½ days p.	—	1	After 6½ days p.	21	1	After 6½ days p.	19
2	" 12½ " p.	19	2	" 8½ " p.	17	2	" 6½ " p.	21	2	" 7 " p.	20½
3	" 14 " d.		3	" 8½ " p.	17	3	" 7 " d.		3	" 7½ " p.	20
4	" 14½ " p.	17	4	" 9 " p.	20½	4	" 7½ " p.	21	4	" 8½ " p.	20
5	" 14½ " p.	—	5	" 9½ " p.	18½	5	" 7½ " p.	—	5	" 8½ " p.	18½
6	" 15 " d.		6	" 9½ " p.	20	6	" 8 " p.	22	6	" 8½ " p.	22
7	" 15 " d.		7	" 10 " d.		7	" 8 " p.	20½	7	" 8½ " p.	20
8	" 15 " d.		8	" 10 " p.	19½	8	" 8 " p.	18½	8	" 8½ " p.	18
9	" 16 " d.		9	" 12½ " p.	16	9	" 8½ " p.	22	9	" 9 " p.	20½
10	" 16 " d.		10	" 12½ " p.	19½	10	" 8½ " p.	—	10	" 9 " p.	17
11	" 16½ " p.	21	11	" 12½ " p.	18	11	" 9 " p.	21½	11	" 9 " p.	18
12	" 17 " d.		12	" 14 " p.	17	12	" 9 " p.	22½	12	" 9½ " p.	18
13	" 17½ " d.		13	" 14 " p.	19	13	" 10½ " p.	21	13	" 9½ " p.	17
14	" 17½ " d.					14	" 11 " d.		14	" 9½ " p.	17
15	" 21½ " d.					15	" 11½ " d.		15	" 10 " p.	20½
16	" 22½ " d.					16	" 12 " p.	17	16	" 10 " p.	19
17	" 22½ " d.								17	" 10 " p.	21½
18	" 23 " d.								18	" 13 " p.	19
19	" 23 " d.										
20	" 23 " d.										
21	" 25 " d.										
22	" 25 " d.										
23	" 27 " d.										
24	" 31 " d.										
25	" 31 " d.										

sidered, but three days later. From Table II. it is evident that here the percentages are quite different: almost all caterpillars undergo pupation. The specimens which did not succeed in becoming pupæ live no longer than the normal larvæ and their death evidently results from the injuries of the operation. In this case the metamorphosis of the caterpillars operated upon does not undergo any retardation.

The above-mentioned experiments of Conte and Vaney ('11) must have been performed on such older specimens. In my previous paper (Kopeć, '12), in which, like the authors mentioned, I stated that the nervous system is unimportant up to the time of pupation, I relied only on full-grown specimens; in view of my present data, the former opinion can no longer be upheld. It follows from further operations that none of the other nervous ganglia (in contrast to the brain) has any influence on the time of pupation.

The dependence of the metamorphosis of the insect on its brain, already discussed, and its independence of the other ganglia, may be observed in one and the same specimen by means of a special method of cutting the caterpillars. Having made ligatures with strong silk round the body of the caterpillar into places chosen *ad libitum,* and having made a section between the ligatures, we may obtain two separate parts from one caterpillar, both of which are able to live. For instance, in one series of experiments the caterpillars were operated upon 7 days after their last moult, and it was found that the parts composed of the head and a few segments of the larval body attained the stage of pupation in from 7 to 9 days after the time of operation. Larger or smaller parts of the hind segments of the body devoid of the brain did not succumb till 35 days after the operation, but at death they exhibited no traces of histolytical processes. When caterpillars a few days older were operated upon both fragments underwent pupation at the normal time.

B. The Nature of the Influence Exerted by the Brain on the Course of Metamorphosis.

If the influence exerted by the brain on the course of metamorphosis were by means of nerves, it might be supposed that the

TABLE II. BEHAVIOR OF THE FEMALE CATERPILLARS OPERATED UPON ON THE TENTH DAY AFTER THEIR LAST MOULT.

p. = pupation; d. = death of caterpillars.

Caterpillars Deprived of the Brain.			Caterpillars Deprived of the Subœsophageal Ganglion (Brain Intact).			Caterpillars Used as a Control; Injuries of the Head as in Caterpillars Deprived of Brain (Brain Intact).			Normal Caterpillars		
Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.	Number Corresponding to Each Specimen.	Behavior of the Caterpillar.	Number of Days the Pupa Lived till the Emergence of the Moth.
1	After 3 days p.	—	1	After 3½ days p.	21	1	After 4 days p.	19½	1	After 3 days p.	20
2	" 3½ " p.	—	2	" 3½ " p.	—	2	" 4 " p.	20½	2	" 4 " p.	21
3	" 4 " p.	21	3	" 4½ " p.	—	3	" 5 " p.	19½	3	" 5 " p.	20
4	" 4 " p.	22	4	" 5 " p.	19½	4	" 5 " p.	19½	4	" 5 " p.	20½
5	" 4 " p.	22	5	" 5 " p.	18½	5	" 5½ " p.	17½	5	" 5 " p.	21
6	" 4 " p.	21	6	" 5 " p.	18½	6	" 6 " p.	—	6	" 5½ " p.	20½
7	" 4 " p.	21	7	" 7 " p.	17½	7	" 6½ " p.	18	7	" 6 " p.	20
8	" 4½ " p.	—	8	" 7 " d.		8	" 8½ " p.	—	8	" 6½ " p.	20½
9	" 5½ " p.	21				9	" 9 " p.	17	9	" 8 " p.	19
10	" 7 " d.					10	" 10½ " d.		10	" 8½ " p.	18½
11	" 8 " d.										
12	" 8½ " p.	—									
13	" 9½ " d.										
14	" 10 " p.	17½									
15	" 10½ " p.	19									

removal of the subœsophageal ganglion would have the same effect as the removal of the brain, as in this case the continuity between the brain and the remainder of the central nervous system is disturbed. But the corresponding data of Tables I. and II. prove that in this case the insects behave normally. The behavior of insects which have been deprived of two or three successive ganglia of the thorax or of the abdomen was also similar: both parts of the body, the part situated in front of the place of operation and that behind it, attained the stage of pupation simultaneously, although the connection between the central nervous system of the two parts had been quite destroyed. After several trials, I convinced myself that processes of regeneration never occur, such as would be able to produce a new nervous communication between the two parts.

At most it must be admitted that the brain is connected with the part of the nervous system situated behind the place of operation by means of the "intestinal nervous system," which is composed of very small ganglia lying in the tissue surrounding the œsophagus and connected in front with the brain. (The "sympathetic nervous system" of the caterpillar undergoes rupture during the removal of the ganglia of the nervous chain. For corresponding anatomical data see the paper of Cattie, '80.) Owing to the unusually small size of the intestinal system, it was impossible to study it in greater detail by operating. On the other hand, I do not consider it possible that any nervous conduction between the two parts of the larval body operated upon can take place, since I observed several times that the part of the body behind the segment deprived of its nervous ganglion does not respond to any stimuli exerted on the fore-part of the caterpillar operated upon, and *vice versa*. The fore-segments of the operated body may be sharply pinched, injured, cut, or burned, still the hind part does not perform even the slightest movements referable to the stimuli, although under normal conditions even slight excitations of any part of the body produce violent reflexes. In regard to this, there seems to be no doubt that the fore-part of the body of the operated caterpillars is in no nervous connection with the part situated beyond the place of operation. It is, therefore, most probable that the brain does not influence the general processes of metamorphosis through the nerves, but that it has rather the function of an organ of internal secretion, in that it affects the organism by means of a substance (or substances) which may be supposed to pass into the blood of the caterpillar from the brain at a certain stage of the larval life. Experiments bearing on the transplantation either of the organized brain or of its matter might perhaps decide whether this conclusion is true. It is most probable that the chemical substance (or substances) here acting is the ferment called "thyrosinase," the aggregation of which during the pupation of insects and the disappearance of which at the end of the pupal life has been previously noticed by Dewitz ('05, '16) and more recently by Steche and Waentig ('13). In his numerous papers Dewitz long ago ascribed an important "rôle" to "thyrosinase" in metamorphotic processes.

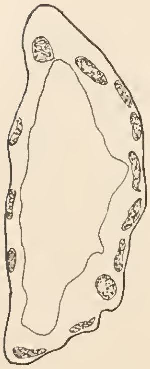
The mere observation that the brain has an influence on the inception of histolytical processes in the body of caterpillars gives no idea as to the physiological nature of this influence. The method of removal of the brain from the larval body was not adapted to ascertain whether the proper stimulus effected by the brain is the only factor which excites histolytical processes in the body of the caterpillar, or whether certain physiological changes which appear in the organism coöperate spontaneously and independently of the brain in the same direction. In other words, does the larval organism undergo metamorphosis exclusively because of the stimulus derived from the brain, or is the brain able to act only when certain physiological changes occur in the tissues of the caterpillar, changes without which the influence of the brain must remain powerless? The investigation in this direction had to be made by transplanting the organs of young caterpillars in which these hypothetical physiological changes in the tissues themselves had not yet occurred onto full-grown caterpillars, in which the influence of the brain on the excitement of metamorphosis might already be noticed. Should the grafted organs exhibit acceleration in their development as a consequence of their new surroundings, the metamorphosis of the insects would have to be considered as dependent primarily on the brain, the latter having caused the transformation of the larval organs independently of their age or physiological development. Should this hypothesis prove false, the coöperation of stimulus of the brain and of the physiological conditions of larval tissues (without which the influence of the brain would have no effect) would appear to be indispensable.

For these transplantations I used the sexual glands. The gonads of moths do not belong to those organs which develop in the chrysalis from the special imaginal discs; they are formed by the growth and histological changes of the small larval sexual glands whose existence has been observed for a long time in caterpillars. All four ovarial tubes of caterpillars hitherto contained in a connective tissue membrane and forming together with it one apparently globular body grow during the caterpillar's pupation to such a degree that they break out of the membrane. In the testes, on the other hand, the characteristic high cylindrical epithelium which lines the interior of that part of the developing spermiduct adjacent to the testicles is formed at that period from the uniform mass of imperfectly separated cells situated at the base of the gland. At the same time the testicle shrinks considerably and its contents become much compacter. I mention these facts in somewhat greater detail than usual in order to emphasize that, although they do not develop from imaginal discs, the sexual glands exhibit certain distinct evolutive changes during their development. As a consequence of this the study of the behavior of the organs after the transplantation is quite suitable for our present purpose. The ovaries and testicles of caterpillars after the third or fourth moult were transferred to full-grown caterpillars which were to undergo pupation in a few days. Hence, in these caterpillars, the substance which excites histolytical processes had already passed over from the brain into the body. It was found that the rate of evolution of the testes and the ovaries grafted remained unchanged, notwithstanding the new surroundings in which the processes of metamorphosis were just beginning or had already begun. From this follows the important conclusion that the stimulus of the brain is inadequate for metamorphosis of the separate organs of insects, and that it is only able to act when the organism, having attained a certain stage of development, is prepared physiologically to respond to the stimulus.

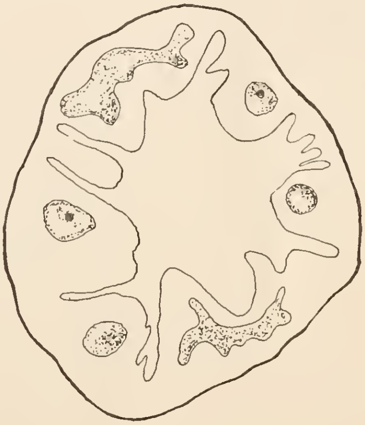
C. The Independence of the Further Processes of Metamorphosis.

I have already mentioned that the brain exerts an influence on the inception of processes of metamorphosis at a certain definite period of the larval life, in females of *Lymantria dispar* L. of my breed between the seventh and tenth day after their last moult. The question arises as to whether this influence, which excites the inception of histolytical processes in caterpillars, at the same time makes the further course of metamorphosis possible, leading to the formation of the imago; or whether brainless chrysalids are unable to become moths. When the caterpillar has been influenced by the brain sufficiently to undergo pupation, the further evolutive processes, which take place in chrysalids, have been shown to occur independently of the brain. By comparing the data relative to the length of life of the chrysalids in various specimens (brainless, those used as standard, and normal) we see, moreover, that even the rate of formation of the imago from the chrysalis undergoes neither retardation nor acceleration when the brain is removed (cf. Tables I. and II.). If a caterpillar deprived of its brain undergoes pupation, the emergence of the moth from the chrysalis takes place at the normal time. The removal of the brain or of other ganglia has merely a local effect in that (as I want to point out in another paper) muscles are completely or almost completely absent in the corresponding segments. The absence of muscles, however, is the result of merely local correlation between the presence of the imaginal muscles and that of the nervous ganglion in a segment of the body, but it has no connection with the influence under discussion—*i.e.,* the influence of the larval brain on the *whole* histolytical and evolutive processes during the metamorphosis of insects in all tissues of the *whole* body.

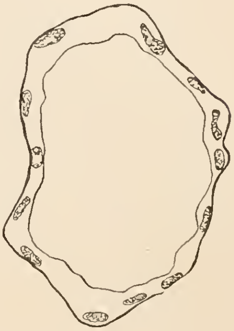
The following experiments were made to demonstrate the above principle, viz., that the further metamorphosis of insects, having once been excited by the influence of the brain, continues independently in a different manner. Evidence ought to be furnished by the metamorphosis of imaginal discs of single parts of the insectal body, if it should take place independently of the surroundings to which they had been artificially transferred. According to recent researches on the development of the Malpighian tubes in moths, the cells of the tubes of full-grown insects are derived directly from the cells of the larval tubes through certain physiological and morphological metamorphosis. (Cf. paper of Samson, '08.) By grafting fragments of the larval Malpighian tubes into the head or thorax, I hoped to solve the problem as to whether the metamorphosis of these tubes might occur normally, in anomalous surroundings, even in the absence of a connection between the tubes and the intestine. Smaller or larger parts of the tubes taken from various regions were grafted into the head or the thorax of the same caterpillar after the last moult of the animal: some of the caterpillars operated upon had just accomplished this moult, others had lived already 10 or 11 days after the last moult. In other cases the Malpighian tubes were transplanted from female caterpillars 10 days after their last moult into other caterpillars 2 or 3 days after this moult. After a few days the ends of these implanted parts were healed up, and so the contents could no longer come out. In the adult insect or pupa I several times succeeded in detecting microscopically the presence of the implanted tubes, which were here not united with the intestine. The cells of the tube which developed in the head or the thorax (cf. Fig. *A* in text) exhibited different characters from those observed in the cater-



A



B



C

pillars (Fig. *B*), viz., they corresponded to the normal cells of tubes in the normal adult moth (Fig. *C*). Their height was far lower than in the larval stage of development, sometimes they were twice as low. Their nuclei, having a much more compact chromatine, and a flatter, non-ramified form, were situated nearer the base of the cells. The interior margin of the tubes was not so much notched as in the caterpillar. All these characters prove that the cells of the implanted tubes have undergone normal metamorphosis. This metamorphosis of the Malpighian tubes is consequently independent of the function of this organ, as it is difficult to suppose that the tubes undergoing metamorphosis in heterogeneous surroundings were able to realize their special function in the chrysalis and later in the adult moth, as they were unconnected with the developing intestine, completely grown together and always filled with excrements previously formed during the larval life. If it could be proved without any objection that the Malpighian tubes which have been implanted into the head or thorax had no nervous junction in the new surroundings, the metamorphosis of transplanted fragments of Malpighian tubes would be a further proof for the assertion made in the previous chapter that the brain exerts its influence by means of internal secretion. The probably unchanged rate of metamorphosis of the tubes transferred from caterpillars 10 days after their last moult into those 2 to 3 days after this moult (thus from specimens in which the influence of the brain was already observable into specimens in which there was yet no such influence) points to the conclusion that tissues once stimulated by the brain to transformation undergo further metamorphosis independently.

Should this principle be of general importance, it would be expected that the organs of much older caterpillars (a few days before pupation) grafted on the much younger caterpillars after the fourth moult (and therefore some two to three weeks younger) might also undergo further metamorphosis at the normal rate, in spite of the fact that the organs of the younger specimens do not as yet indicate the slightest trace of the histolytical processes. Transplantations of the germ of wings fully confirmed this hypothesis. I removed the germ of the first left wing from numerous caterpillars shortly before their last moult, and in its place I grafted an analogous germ derived from a full-grown caterpillar, which was to undergo metamorphosis in a few days. In two of the caterpillars somewhat large and black outgrowths—dwarfed and deformed wings—appeared on the place of grafting. In their uniformly dark hue and the thickness and markings of the chitin they formed a striking contrast to the bright-colored tegument of the caterpillar. (Cf. Fig. *D*.) The small size and the abnormal forms were doubtless caused by inevitable injuries of the delicate germ during transplantation, as well as by the difficulty of extracting the pupal wing from the moulting but not pupating caterpillar. Histologically the development of the pupal wings was normal. I have also formerly observed (Kopeć, '11) that the sexual glands of full-grown caterpillars develop at their normal rate when grafted on the caterpillar after their third or fourth moult. Such a behavior of the grafted wings and sexual glands strikingly confirmed the above-stated principle that after the time when the brain has already exerted its influence on the whole organism—which influence decides the inception of histolytical processes leading to the chrysalis stage—the subsequent development and metamorphosis of organs is quite independent of the brain.

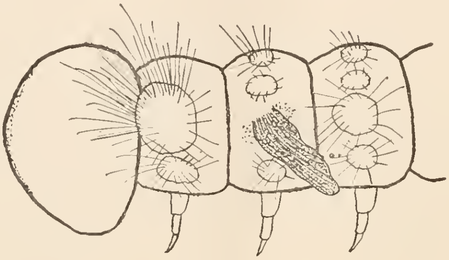


Fig. *D*.

The results of my experiments proved that the rate of development of the grafted organs underwent no change due to the influence of new surroundings, differing both as to age and physiological state. These results seem to disagree with analogous researches of Uhlenhuth ('12, '13) on amphibians. This author grafted eyes of salamander larvæ on specimens of a different age and came to the conclusion that the metamorphosis of the transplanted eyes underwent a distinct retardation when grafted on younger specimens, and an acceleration in the contrary case—*i.e.*, the transplanted as well as the own eyes of the foster-mother underwent metamorphosis at the same time. But the disagreement of these results with the behavior of the transplanted insect organs in my investigations is only apparent. The influence of the surroundings on the grafted eye, observed by Uhlenhuth, was to be remarked only when the age of the specimens on which the eyes were implanted did not differ much from that of animals whose eyes were taken for the operation. In the remaining cases—*i.e.*, under the conditions which alone corresponded to my investigations on the transplantation of organs from young caterpillars after their third or fourth moult to full-grown caterpillars or chrysalids and *vice versa*—the grafted eyes retained their proper developmental rate without being influenced by the new surroundings, just like the organs of insects transplanted in my experiments. Nor do my experiments on insects in any way contradict the experiments of Weigl ('13) which were made simultaneously with but independently of those of Uhlenhuth. Weigl studied the same problem in his investigations on the homo- and heteroplastic transplantation of the skin in salamanders and other amphibians. In regard to the course of the metamorphosis of the grafted pieces of skin, he comes to the same general conclusion as Uhlenhuth. In the experiments of Kornfeld ('14) we can also find a parallel to the results I observed on insects. This author, who transplanted the gill of salamanders, agrees in his final conclusion with Uhlenhuth. Uhlenhuth ('13), mentioning my former experiments on the transplantation of gonads in moths, imputes to me results completely different from those which I really obtained (Kopeć, '11). More recently this error has been corrected by Kornfeld in agreement with Uhlenhuth.

In specimens deprived of the brain or of other nervous ganglia we often may observe certain mechanical difficulties during pupation and emergence of the moth. They are produced by concrescence of the chitin at the place of operation, and cause the appearance of certain deformities of chrysalids or of moths. It must be distinctly noted that all these deformities, caused only by mechanical obstructions during pupation or emergence of the moths, are in no wise dependent on the operation itself, nor consequently on the removal of separate parts of the nervous system. A proof of this is that they also appear—and in the same number of cases—in the specimens with intact nervous system used as a standard and injured similarly as in the operations on the ganglia. Therefore I consider it superfluous to describe these anomalies here.

D. General Conclusions and Summary.

From the whole of this paper it follows that my results relative to the influence of the nervous system on metamorphosis in insects show a very great resemblance to the relations observed in amphibians. According to recent investigations (above mentioned), iodin stored by the thyroid glands in amphibians is the direct cause which elicits metamorphosis, but the production of this substance probably depends on the function of the hypophysis. Consequently in both classes of animals metamorphosis would be controlled by the brain, or part of it. In recent years experiments have been performed on the influence of food containing thyroid or hypophysis on the development of the larvæ of insects, but the results of these researches do not as yet furnish an adequate material for any conclusions. Abderhalden ('19) observed that a part of the moths obtained from caterpillars fed on hypophysis was very large; many specimens from caterpillars fed on thyroid, on the contrary, were very small. These data refer, however, to phenomena of growth, and are possibly quite unrelated to the problem of the rate of transformation here now discussed. Romeis and v. Dobkiewicz ('20) studied the rate of development in larvæ of *Calliphora* fed on thyroid and obtained no distinct changes which might have been attributed exclusively to the specific effect of the thyroid gland. As I mentioned above, negative results of feeding experiments with hypothetical specific influence ought not to be considered as decisive, because it may be inferred from them only that the substance used does not exert any effect on the organism when given *per os*. Hankó ('12), on the other hand, ascertained a very considerable influence of hypophysis on rate of moults in *Asellus aquaticus*. But, as moults in Crustacea are not to be totally identified with moults in insects having complete metamorphosis, these results also have no great importance for our problem. The whole question demands further research in order to elucidate whether and in what degree there exists an analogy between the physiology of transformation of amphibians and that of insects.

The following summary contains the more important results of this paper:

1. The brain (ganglion supra-œsophageale) of the caterpillar of *Lymantria dispar* L. has particular importance in the general processes of metamorphosis. The presence of the brain is indispensable, at least up to a certain period, for the inception of histolytical processes. The influence of the brain in this direction is probably chemical; hence the brain ought to be considered as a gland of internal secretion.

2. At some well-defined time before pupation the quantity of the corresponding substance (or substances) secreted by the brain is already sufficient for the complete pupation of the caterpillar.

3. Tissues of the caterpillar influenced by the brain undergo further metamorphosis independently; the germs of the wings of caterpillars grafted shortly before pupation on younger caterpillars near their last moult are transformed in the larval organism into pupal wings in spite of the absence of histolytical processes in the new surroundings. Analogical results have been obtained by transplantation of Malpighian tubes and gonads.

4. It follows from other experiments on the transplantation of organs that the stimulus coming from the brain is not sufficient by itself for the metamorphosis of caterpillars: this stimulus acts only when the insect organism, having attained a certain stage, is physiologically prepared to answer to this influence. This is shown by the fact that germs of gonads from young caterpillars grafted into full-grown caterpillars do not undergo metamorphosis characteristic of the pupal development of gonads; in other words, the rate of development underwent no acceleration in spite of the histolytical processes occurring in the new surroundings.

5. Other parts of the nervous system have no influence on the general processes of metamorphosis.

6. By making ligatures round the body of the caterpillar and cutting it in different places, we may obtain fragments consisting of front, middle, or hind segments. The front fragments undergo

metamorphosis in a normal manner, provided the caterpillar does not die of starvation. On the contrary, the middle and hind fragments undergo pupation only if they have belonged to caterpillars which would have pupated in a few days.

7. The Malpighian tubes are transformed into tubes of the adult insect independently of the intestine and of the performance of their specific function.

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